



VETERINARY IMMUNOLOGY

AN INTRODUCTION

EIGHTH EDITION

Ian R. Tizard

Veterinary Immunology: An Introduction, 8th Edition

Veterinary Immunology

vi

AN INTRODUCTION

8th ed.

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Front Matter

Dedication

To Devon and Trevor

Abbreviations

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ADCC

antibody-dependent cell-mediated cytotoxicity

ADP

adenosine diphosphate

AIDS

acquired immune deficiency syndrome

AIHA

autoimmune hemolytic anemia

AITP

autoimmune thrombocytopenia

ANA

antinuclear antibody

APC

antigen-presenting cell

ATP

adenosine triphosphate

BALT

bronchus-associated lymphoid tissue

BCG

bacillus Calmette-Guérin (*Mycobacterium bovis*)

BCR

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B cell antigen receptor

BHV

bovine herpesvirus

BLAD

bovine leukocyte adhesion deficiency

BLV

bovine leukemia virus

BoLA

bovine leukocyte antigen

BVDV

bovine viral diarrhea virus

C

complement

CAM

cell adhesion molecule

CBH

cutaneous basophil hypersensitivity

CD

cluster of differentiation

CDR

complementarity determining region

CDw

cluster of differentiation (provisional designation)

CFT

complement fixation test

CID

combined immunodeficiency

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CLL

chronic lymphoid leukemia

Con A

concanavalin A

COX

cyclooxygenase

CpG

cytosine-guanosine

CR

complement receptor

CRP

C-reactive protein

CSF

colony stimulating factor (or cerebrospinal fluid)

DAF

decay accelerating factor

DAG

diacylglycerol

DAMP

damage-associated molecular pattern

DC

dendritic cell

DEA

dog erythrocyte antigen

dsRNA

double-stranded RNA

DTH

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delayed-type hypersensitivity

EAE

experimental allergic encephalitis

EAF

eosinophil activating factor

EAN

experimental allergic neuritis

ECF

eosinophil chemotactic factor

ELISA

enzyme-linked immunosorbent assay

EPO

eosinophil peroxidase

Fab

antigen-binding fragment

Fc

crystallizable fragment (of immunoglobulin)

FCA

Freund's complete adjuvant

FcR

Fc receptor

FeLV

feline leukemia virus

FITC

fluorescein isothiocyanate

FIV

feline immunodeficiency virus

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FOCMA

feline oncornavirus cell membrane antigen

FPT

failure of passive transfer

GALT

gut-associated lymphoid tissue

GDP

guanosine diphosphate

GM-CSF

granulocyte-macrophage colony-stimulating factor

GPI

glycosyl-phosphatidylinositol

GTP

guanosine triphosphate

GVH

graft-versus-host (disease)

HAT

hypoxanthine aminopterin thymidine (medium)

HDN

hemolytic disease of the newborn

HEV

high endothelial venule

HI

hemagglutination inhibition

HIV

human immunodeficiency virus

HLA

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human leukocyte antigen

HSP

heat-shock protein

Ia

mouse class II MHC molecule

ICAM

intercellular adhesion molecule

IDDM

insulin-dependent diabetes mellitus

IEL

intraepithelial lymphocytes

IFA

indirect fluorescence assay

IFN

interferon

Ig

immunoglobulin

IK

immuncongglutinin

IKK

IκB kinase

IL

interleukin

IMHA

immune-mediated hemolytic anemia

IRAK

interleukin receptor-associated kinase

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ISCOM

immune-stimulating complex

ISG

immune serum globulin

ITAM

immunoreceptor tyrosine-based activation motif

ITP

inositol triphosphate

IU

international unit

J

joining

kDa

kilodalton

LAD

leukocyte adhesion deficiency

LAK

lymphokine-activated killer (cells)

LBP

lipopolysaccharide binding protein

LD₅₀

lethal dose 50

LE

lupus erythematosus

LFA

leukocyte function-associated antigen

LGL

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large granular lymphocyte

lpr

lymphoproliferation

LPS

lipopolysaccharide

LT

lymphotoxin (or leukotriene)

β_2 M

β_2 -microglobulin

MAC

membrane attack complex

MASP

MBL-associated serine protease

MBL

mannose-binding lectin

M-CSF

macrophage colony-stimulating factor

MG

myasthenia gravis

MHC

major histocompatibility complex

MIP

macrophage inflammatory protein

MLC

mixed lymphocyte culture

MLD

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minimal lethal dose

MLR

mixed lymphocyte reaction

MLV

modified live virus

MPGN

mesangioproliferative glomerulonephritis

NF- κ B

nuclear factor kappa-B

NK

natural killer (cell)

NKT

natural killer T cell

NO

nitric oxide

NOD

nucleotide-binding oligomerization domain

NOS

nitric oxide synthase

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NOX

NADPH oxidase

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NS

natural suppressor (cell)

OAS

oligoadenylate synthetase

PAF

platelet-activating factor

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PAMP

pathogen-associated molecular pattern

PCA

passive cutaneous anaphylaxis

PF

protective fraction

PFC

plaque-forming cell

PG

prostaglandin

PHA

phytohemagglutinin

pIgR

receptor for polymeric immunoglobulin

PKC

protein kinase C

PLC

phospholipase C

PPD

purified protein derivative of tuberculin

PWM

pokeweed mitogen

R

receptor (e.g., IL-2R)

RAST

radioallergosorbent test

RF

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rheumatoid factor

RIA

radioimmunoassay

RIG

retinoic acid inducible gene

S19

strain 19 *Brucella abortus* vaccine

SAA

serum amyloid A (protein)

SAP

serum amyloid P

SCID

severe combined immunodeficiency

SID

single intradermal test

SLA

swine leukocyte antigen

SLE

systemic lupus erythematosus

SMAC

supramolecular activation cluster

ssRNA

single-stranded RNA

STAT

signal transducer and activator of transcription

TAP

transporter for antigen processing

TB

tuberculosis

TCID₅₀

tissue culture infective dose 50

TCR

T cell antigen receptor

Tcs cell

contrasuppressor T cell

TdT

terminal deoxynucleotidyl transferase

TGF

transforming growth factor

Th cell

helper T cell

TIL

tumor infiltrating lymphocytes

TLR

toll-like receptor

TK

thymidine kinase

TNF

tumor necrosis factor

TRAF

tumor necrosis factor receptor-associated factor

T_{reg} cell

regulatory T cell

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VCAM

vascular cell adhesion molecule

VKH

Vogt-Koyanagi-Harada (syndrome)

VLA

very late antigen

WC

workshop cluster

ZAP

zeta-associated protein

Greek Letters

Lower case Greek letters are widely used in immunology to denote peptide chains or other molecules. Following is a list of Greek letters with examples of their usage.

Examples of Greek Letters in Immunology

α alpha

α heavy chains (IgA)

β beta

β_2 -microglobulin

γ gamma

γ globulin, γ interferon

δ delta

δ heavy chains (IgD)

ϵ epsilon

ϵ heavy chains (IgE)

ζ zeta

ζ chain of CD3

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η eta

η chain of CD3

θ theta

θ antigen (a synonym for CD90)

κ kappa

κ light chains

λ lambda

λ light chains

μ mu

μ heavy chains (IgM)

υ upsilon

υ heavy chain (IgY)

φ phi

φX174, a bacteriophage

ψ psi

a notation for a pseudogene

τ tau

interferon-τ

ω omega

interferon-ω

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Preface

It is now 30 years since the first edition of this text was written. Veterinary immunology has changed immeasurably since the 1970s. That decade saw the first flowering of the discipline as the new techniques of molecular biology were applied to the immune systems of domestic animals. Over the intervening years, many details have been added to our knowledge of basic immunological processes. Many of the major questions of the 1970s have been answered. Much of what remains to be done involves the detailed clarification of pathways and mechanisms already known in broad outline. Nevertheless, significant gaps remain to be investigated. For example, we still know remarkably little about species differences and the reasons why the immune system functions differently in different species. Most immunologists remain content to study the immune systems of mice or humans. Even veterinarians seem content to

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focus exclusively on the major domestic animal species. Astonishingly little is known about the immune system in most nondomesticated mammals.

The revolutions in genomics and molecular biology have, among other achievements, generated a broadly accepted mammalian phylogeny. There is thus a much clearer understanding of the evolutionary relationships between the major groups of mammals. Even the less obvious relationships such as those between the cetaceans (whales and dolphins) and the artiodactyls (cattle) have been firmly established. Despite this, mammalian immunology still fails to be truly comparative. Ever since the first edition of this book I have been concerned about the lack of discernible patterns in the structure and function of the immune system between different mammalian species. Why do some species have pulmonary intravascular macrophages while others do not? Why do some species rely on the intestinal microflora for B cell development while others do not? I strongly suspect that hidden within in the structure and function of mammalian immune systems are logical patterns that remain to be identified. Consider the immunological challenges faced by herbivores and carnivores. The latter are much more likely to be exposed to mammalian pathogens through eating dead or dying prey. Surely this would affect the way in which their immune systems have evolved. If we are to discern these differences, I believe it essential that veterinary immunologists focus much more closely than hitherto on the comparative aspects of the immune system. The recent completion of whole genome sequencing of many mammals and the imminent sequencing of others will provide an unrivalled opportunity to compare immune system genes and identify patterns that relate directly to the functioning of immune systems in all mammals. As a small gesture of support of this phylogenic approach, I have ensured that domestic mammal species are listed in phylo-genetic order throughout this new edition: horse/cattle/sheep/pig/dog/cat/mouse/human.

ORGANIZATION

The overall design of this text is basically unchanged from previous editions. The first half of the book seeks to provide a review of basic immunology. The second half encompasses applied veterinary immunology. The first half must therefore be considered a review, not a comprehensive reference text, and I make no pretence that it is complete in all details. My intention is to provide the student of veterinary medicine (both before and after graduation) with a basis for understanding the applied aspects of the subject. Three major chapter changes have been made in an effort to improve the learning process; thus the chapter on complement has been moved to the section on innate immunity in recognition of its critical importance as an innate defense mechanism and as an important trigger of inflammation. The chapter on cytokines, with its long list of such molecules, has been replaced with a chapter dealing with cytokines in general, cytokine receptors, and cell signaling. It has been placed early in the chapter sequence ([Chapter 6](#)) as a basic science chapter. Those of you who yearn for a long list of cytokines can find it in a new appendix. The chapter on serology and serological techniques has been moved to the back of the book, not because of its lack of importance, but because serology is often taught separately from the main lecture series in a laboratory setting.

The past four years have seen few dramatic advances in veterinary immunology. Nevertheless, DNA vaccines and feed-based vaccines have now entered the market. The debate on the duration of immunity and vaccination intervals has declined in intensity. Scientifically, much longer intervals between vaccine doses are justified. Experience, however, shows that the majority of practicing veterinarians have retained their policy of annual revaccination.

Arguably the most significant advances in veterinary immunology over the past four years have been the studies of Dr. Larry Glickman and his colleagues on the prevalence of vaccine-associated adverse events in dogs and cats. Using an enormous database containing details of several million events from a major chain of veterinary hospitals, Dr. Glickman has arrived at by far the most reliable estimate for the prevalence of these adverse events.

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This prevalence is approximately 10 times the figure obtained by voluntary reporting. At the same time, he and his colleagues have shown that small dogs are at much greater risk of these adverse events than larger dogs and have brought into question the practice of giving the same dose of vaccine to animals regardless of size. Few new immunological diseases have been identified, although the continuing practice of inbreeding domestic pets continues to ensure a steady supply of genetic diseases to interested researchers.

Among the other topics described in this edition is new information on innate immunity. For example, much more information is now available on toll-like receptors and their central role in triggering both innate and acquired immunity. It appears that these receptors are not only central to innate immunity but play a key role in regulating B cell responses. New information on the receptors of natural killer (NK) cells is also included, since there are major species differences in the way in which NK cells recognize their targets. Finally, the subject of antibacterial peptides is greatly expanded as we come to recognize their importance in inflammation and innate immunity. One other item that may well assume greater importance in the future is the growing awareness that cells freely exchange membranes. As a result, for example, bovine neutrophils may obtain patches of surface membranes together with their associated receptors from macrophages. This may have significant effects on their function.

In the area of acquired immunity, new developments include an increased understanding of the remarkable differences in immunoglobulin D (IgD) between species. Rabbits remain the only major mammal in which IgD has not been identified. It is not known whether this is real or simply a matter of looking harder. Many more new cytokines have been identified as have huge numbers of new CD molecules. It is tempting to suggest that the most recent molecules identified are of less significance than those described previously, but only time will tell. That said, however, it is clear that the existence of a third population of helper T cells, Th17 cells, and their activities through the IL-23/IL-17 axis can readily explain many hitherto complex situations. For example, Th17 cells appear to be critical for resistance to fungi, and they also appear to play the key role in the pathogenesis of rheumatoid arthritis. There is much new information on the phylogeny of the immune system, and new fish immunoglobulins are being identified at a regular pace. The feline major histocompatibility complex has been mapped. New IgA receptors have been identified. The signals that determine the development of different macrophage phenotypes have been clarified. However, just to ensure that we don't get too complacent, it has recently been shown that mouse neutrophils express T cell antigen receptors!

In the area of applied antimicrobial resistance, some of the most interesting recent information has come from identification of the mechanisms that ensure that commensal bacteria neither invade the intestinal wall nor trigger uncontrolled inflammation. Likewise there is much new information on immunosenescence and the decline in immune function as dogs and cats age. New data on the functions of γ/δ T cells, on the effects of nematodes on eosinophils, and on some of the newer DNA vaccines against West Nile virus and canine melanoma are also provided.

In the area of immunological diseases we have new information on the heritability of atopic disease. "New" diseases such as Stevens-Johnson syndrome, hemophagocytic syndrome, and autoimmune sarcolemmal disease in dogs, as well as common variable immunodeficiencies and immune-mediated polysynovitis in horses, are described. Information is also provided on the mechanisms of action of the newer immunosuppressive drugs and their use in diseases of domestic animals.

Finally, readers are strongly encouraged to visit the Evolve website for this text. While this website is constantly evolving and improving, you will be able to find a collection of more than 450 multiple choice questions (with the answers) keyed to each chapter; all the text figures (available for downloading); a small collection of animations kindly provided by Dr. Abdul Abbas; and all the chapter references keyed to PubMed. You will also find a monthly update of new information gleaned from the current veterinary immunology literature. This too is

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arranged according to the book chapters and will ensure that you remain current in this rapidly expanding and exciting field.

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Acknowledgments

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Writing a book takes away, of course, from the time allocated to other tasks. I am especially grateful to my assistant, Debra Turner, who keeps my research program functioning when I am irreversibly immersed in writing.

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Ian Tizard

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¹ CHAPTER 1 The Defense of the Body

1.1 KEY POINTS

- The immune system protects animals against microbial invasion and is therefore essential for life.
- Multiple mechanisms are needed to ensure freedom from disease. These include physical barriers that exclude invaders, innate immunity that provides rapid initial protection, and acquired immunity that provides prolonged effective immunity.
- One form of acquired immunity is mediated by antibodies. Antibodies protect against extracellular invaders such as bacteria. Antibodies circulate in body fluids, especially in the bloodstream.
- Another form of acquired immunity is cell-mediated immunity. This protects animals against intracellular invaders such as viruses.
- The acquired immune system has the ability to remember prior exposure to foreign invaders and mount a faster and more effective response on subsequent exposure to an invader. This effectively ensures an animal's survival in the face of continuing microbial challenge.

The animal body contains all the components necessary to sustain life. It is warm, moist, and rich in many different nutrients. As a result, animal tissues are extremely attractive to microorganisms that seek to invade the body and exploit these resources for themselves. The magnitude of this microbial attack can be readily seen when an animal dies. Within a few hours, especially when warm, a body decomposes rapidly as microbes invade its tissues. On the other hand, the tissues of living, healthy animals are highly resistant to microbial invasion. Indeed, the survival of an animal depends on its successful defense against microbial invaders. This resistance is due to multiple interlinked defense mechanisms. The defense of the body is encompassed by the discipline of immunology and is the subject of this book.

Because effective resistance to infection is critical, the body dare not rely on a single defense mechanism. To be effective and reliable, multiple defense systems must be available. Some may be effective against many different invaders. Others may only destroy specific organisms. Some act at the body surface to exclude invaders. Others act deep within the body to destroy organisms that have breached the outer defenses. Some defend against bacterial invaders, some against viruses that live inside cells, and some against even large invaders such as fungi or parasitic worms and insects. The protection of the body comes from a complex system of overlapping and interlinked defense mechanisms that together can destroy or control almost all invaders. A failure in these defenses either because the immune system is destroyed (as occurs in acquired immune deficiency syndrome; AIDS) or because the invading organisms can overcome or evade the defenses will result in disease and possibly death. An effective immune system is not simply a useful system to have around. It is essential to life itself.

1.2 A BRIEF HISTORY OF VETERINARY IMMUNOLOGY

Our awareness of the importance of the defense of the body against microbial invasion could only develop after the medical community accepted the concept of infectious disease. When infections such as smallpox or plague spread through early human societies, many people died, but some individuals recovered. It was rarely noticed that these recovered individuals remained healthy during subsequent outbreaks—a sign that they had developed effective immunity. Nevertheless, by the 12th century the Chinese had observed that those individuals who recovered from

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smallpox were resistant to further attacks of this disease. Being practical people, they deliberately infected infants with smallpox by inserting scabs from infected individuals into small cuts in their skin. Those infants who survived the resulting disease were protected from smallpox in later life. The risks inherent in this procedure were acceptable in an era of high infant mortality. On gaining experience with the technique it was found that using scabs from the mildest smallpox cases minimized the hazards. As a result, mortality due to smallpox inoculation (or variolation) dropped to about 1% as compared to a mortality of about 20% in clinical smallpox cases. Knowledge of variolation spread westward to Europe by the early 18th century, and the practice was soon widely employed.

Outbreaks of rinderpest (then called cattle plague) had been a common occurrence throughout Western Europe since the ninth century and inevitably killed huge numbers of cattle. Since none of the traditional remedies appeared to work and the skin lesions in affected animals vaguely resembled those seen in smallpox, it was suggested in 1754 that inoculation might help. This process involved soaking a piece of string in the nasal discharge from an animal with rinderpest and then inserting the string into an incision in the dewlap of the animal to be protected. The resulting disease was usually milder than natural infection, and the inoculated animal became resistant to the disease. The process proved very popular, and skilled inoculators traveled throughout Europe inoculating cattle and branding them to show that they were protected against rinderpest.

In 1798, Edward Jenner, an English physician, demonstrated that material from cowpox lesions could be substituted for smallpox in variolation. Since cowpox does not cause severe disease in humans, its use reduced the risks incurred by variolation to insignificant levels. The effectiveness of this procedure, called vaccination (*vacca* is Latin for “cow”) was such that it was eventually used in the 1970s to eradicate smallpox from the world.

Once the general principles of inoculation were accepted (even though nobody had the faintest idea how it worked), attempts were made to use similar procedures to prevent other animal diseases. Some of these techniques were effective. Thus material derived from sheep pox was used successfully to protect sheep in a process called ovination and was widely employed in Europe. Likewise, inoculation for bovine pleuropneumonia consisted of inserting a small piece of tissue from an infected lung into a cut in the tail. The tail fell off within a few weeks, but the animal became immune! Although the process was effective, infected material from the tail also spread the disease and so delayed its eradication. On the other hand, administration of cowpox scabs to the nose of puppies to prevent canine distemper, though widely employed, was a complete failure.

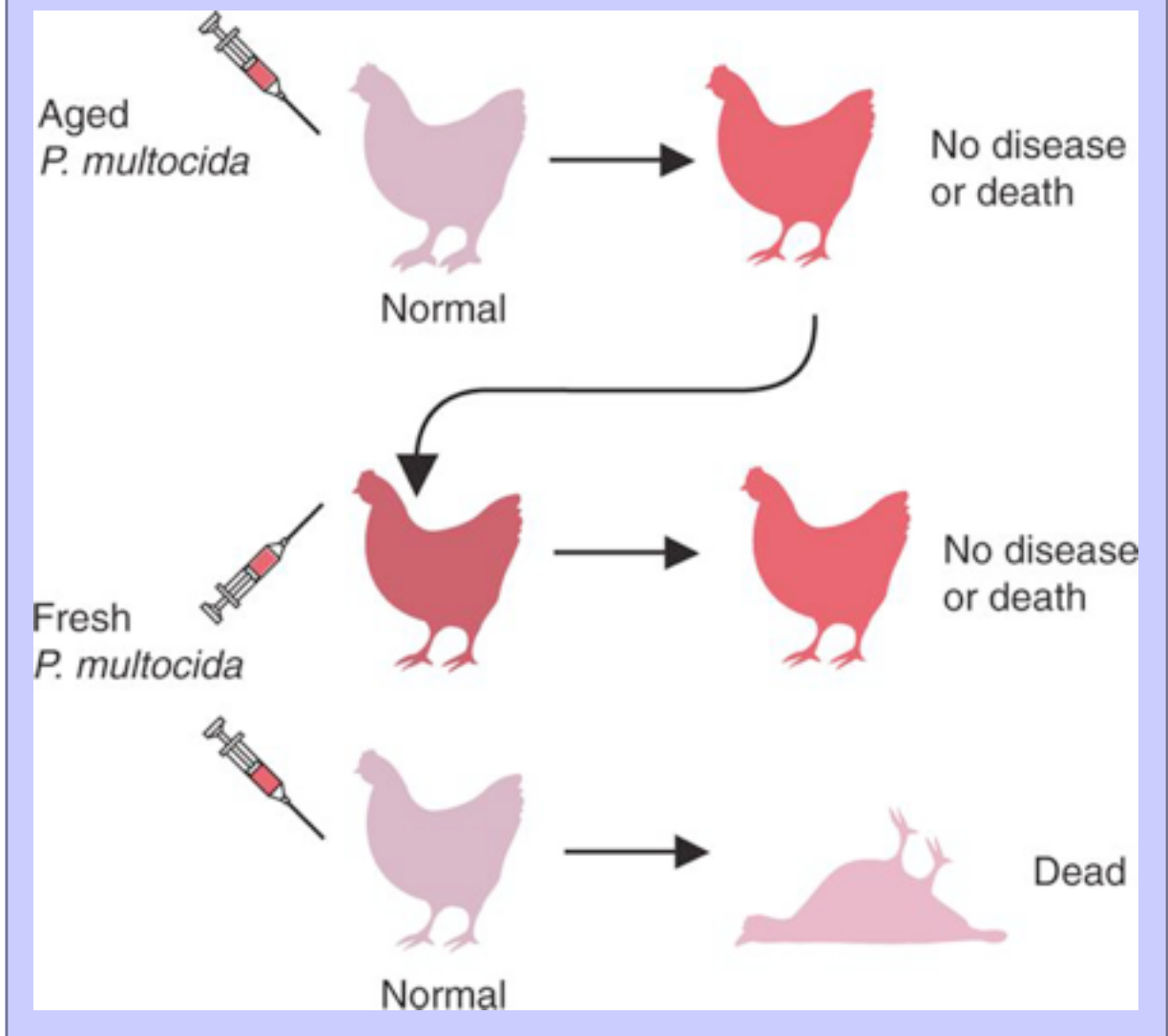
The general implications of Jenner's observations on cowpox and the importance of reducing the ability of an immunizing organism to cause disease were not realized until 1879. In that year, Louis Pasteur in France investigated fowl cholera, a disease caused by the bacterium now called *Pasteurella multocida* ([Figure 1-1](#)). Pasteur had a culture of this organism that was accidentally allowed to age on a laboratory bench while his assistant was on vacation. When the assistant

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FIGURE 1-1 Louis Pasteur made the key discoveries that led to the development of vaccines against infectious agents. This drawing shows him as the good shepherd “Le bon Pasteur,” reflecting his discovery of a vaccine against anthrax, 1882. (Copyright Institut Pasteur. With permission.)



FIGURE 1-2 Pasteur's fowl cholera experiment. Birds inoculated with an aged culture of *Pasturella multocida* did not die. However, when subsequently inoculated with a fresh culture of virulent *P. multocida*, the birds were found to be protected.



returned and tried to infect chickens with this aged culture, the birds remained healthy ([Figure 1-2](#)).

Being frugal, Pasteur retained these chickens and subsequently used them for a second experiment in which they were challenged again, this time with a fresh culture of *P. multocida* known to be capable of killing chickens. To Pasteur's surprise the birds were resistant to the infection and did not die. In a remarkable intellectual jump, Pasteur immediately recognized that this phenomenon was similar in principle to Jenner's use of cowpox for vaccination. In vaccination, exposure of an animal to a strain of an organism that will not cause disease (an avirulent strain) can provoke an immune response. This immune response will protect the animal against a subsequent infection by a disease-producing (or virulent) strain of the same, or closely related, organism. Having established the general

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principle of vaccination, Pasteur first applied it to anthrax. He made anthrax bacteria (*Bacillus anthracis*) avirulent by growing them at an unusually high temperature. These attenuated organisms were then used as a vaccine to protect sheep against challenge with virulent anthrax bacteria. Pasteur subsequently developed a successful rabies vaccine by drying spinal cords taken from rabies-infected rabbits and using the dried cords as his vaccine material. The drying process effectively rendered the rabies virus avirulent (and probably killed much of it).

Although Pasteur used only living organisms in his vaccines, it was not long before Daniel Salmon and Theobald Smith, working in the United States, demonstrated that dead organisms could also be used as vaccines. They showed that a heat-killed culture of a bacterium called *Salmonella enterica choleraesuis* (then called *Bacillus suispestifer* and believed to be the cause of hog cholera) could protect pigeons against the disease caused by that organism. A little later, Emil Von Behring and Shibasaburo Kitasato in Germany showed that filtrates taken from cultures of the tetanus bacillus (*Clostridium tetani*) could protect animals against tetanus even when they contained no bacteria. Thus bacterial products, in this case tetanus toxin, were also protective.

By 1900 the existence of immunity to infectious diseases of animals was well recognized. Since then, immunologists have succeeded in identifying the molecular and cellular basis of this antimicrobial immunity. With this understanding has come the ability to use immune mechanisms to enhance resistance to infectious diseases. The role of the immune system in many different disease processes has been clarified. While much has been learned, much remains to be investigated. The current state of immunology as it relates to species of interest to veterinarians is the subject of this book.

1.3

MICROBIAL INVASION

The world is full of a diverse array of microorganisms. These include bacteria, viruses, fungi, protozoa, and helminths (worms). As they struggle to survive, many of these microorganisms see the animal body as a rich source of nutrients and a place to shelter. They will thus seek to invade animal tissues. This is normally prevented, or at least controlled, by our immune defenses. If these organisms overcome the immune defenses, they may cause disease. Some organisms have evolved to successfully invade the animal body. These infectious agents can only survive if they avoid the host's immune system for sufficient time to replicate and transmit their progeny to a new host. While it is essential for an animal to control infectious agents, the infectious agent is under even more potent selective pressure. It must find a host or die. Organisms that cannot evade or overcome the immune defenses will not survive within the body and will be eliminated.

An organism that can cause disease is called a pathogen. It is important to point out, however, that only a small proportion of the world's microorganisms are associated with animals and that only a very small proportion of these have the ability to overcome the immune defenses and become pathogens.

Microorganisms vary greatly in their ability to cause disease (or to evade the body's defenses). This ability is termed virulence. Thus a highly virulent organism has a greater ability to defeat the immune system and cause disease than an organism with low virulence. If a bacterium can cause disease almost every time it invades a healthy individual, even in low numbers, then it is considered a primary pathogen. Examples of primary pathogens include canine distemper virus; the human immunodeficiency virus (HIV), which causes AIDS; and *Brucella abortus*, the cause of contagious abortion in cattle. Other pathogens may be of such low virulence that they will only cause disease if administered in very high doses or if the immune defenses of the body are impaired first. These are opportunistic pathogens. Examples of opportunistic pathogens include bacteria such as *Mannheimia hemolytica* and fungi such as *Pneumocystis carinii*. These organisms rarely, if ever, cause disease in healthy animals.

3

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1.4 THE BODY'S DEFENSES

1.4.1 Physical Barriers

The most effective defenses of the body involve denial of access. Without such defensive barriers, successful defense is almost impossible. Because the successful exclusion of microbial invaders is essential for survival, it is not surprising that animals use many different defense strategies. Indeed, the body employs multiple, overlapping layers of defense ([Figure 1-3](#)). As a result, an organism that has succeeded in breaking through the first defensive layer is then confronted with the need to overcome a second, higher barrier, and so forth. The first and most obvious of these layers are the physical barriers to invasion. For example, intact skin provides an effective barrier to microbial invasion. If it is damaged, infections may occur; however, wound healing ensures that the barrier is restored very rapidly. On other body surfaces, such as in the respiratory and gastrointestinal tracts, simple physical defenses include the “self-cleaning” processes: coughing, sneezing, and mucus flow in the respiratory tract; vomiting and diarrhea in the gastrointestinal tract; and urine flow in the urinary system. The presence of an established normal flora on the skin and in the intestine also excludes many potential invaders. Well-adapted commensal organisms adapted to living on body surfaces can easily outcompete poorly adapted pathogenic organisms.

1.4.2 Innate Immunity

Physical barriers, though essential in excluding in-vaders, cannot be totally effective in themselves. Given time and persistence, an invading microorganism will eventually overcome mere physical obstacles. Animals are not, however, perpetually sick, presumably because most microbial attempts at invasion are blocked before they can result in disease. This is the task of the innate immune system. This second layer of defenses therefore consists of rapidly responding chemical and cellular defense mechanisms ([Table 1-1](#)). Innate immunity relies on the fact that invading microorganisms differ chemically from normal body components. Thus animals have enzymes that can digest bacterial cell walls and carbohydrate-binding proteins that will coat bacteria and hasten their destruction. Animals also have cells that can recognize the molecules commonly associated with invading microorganisms and kill them.

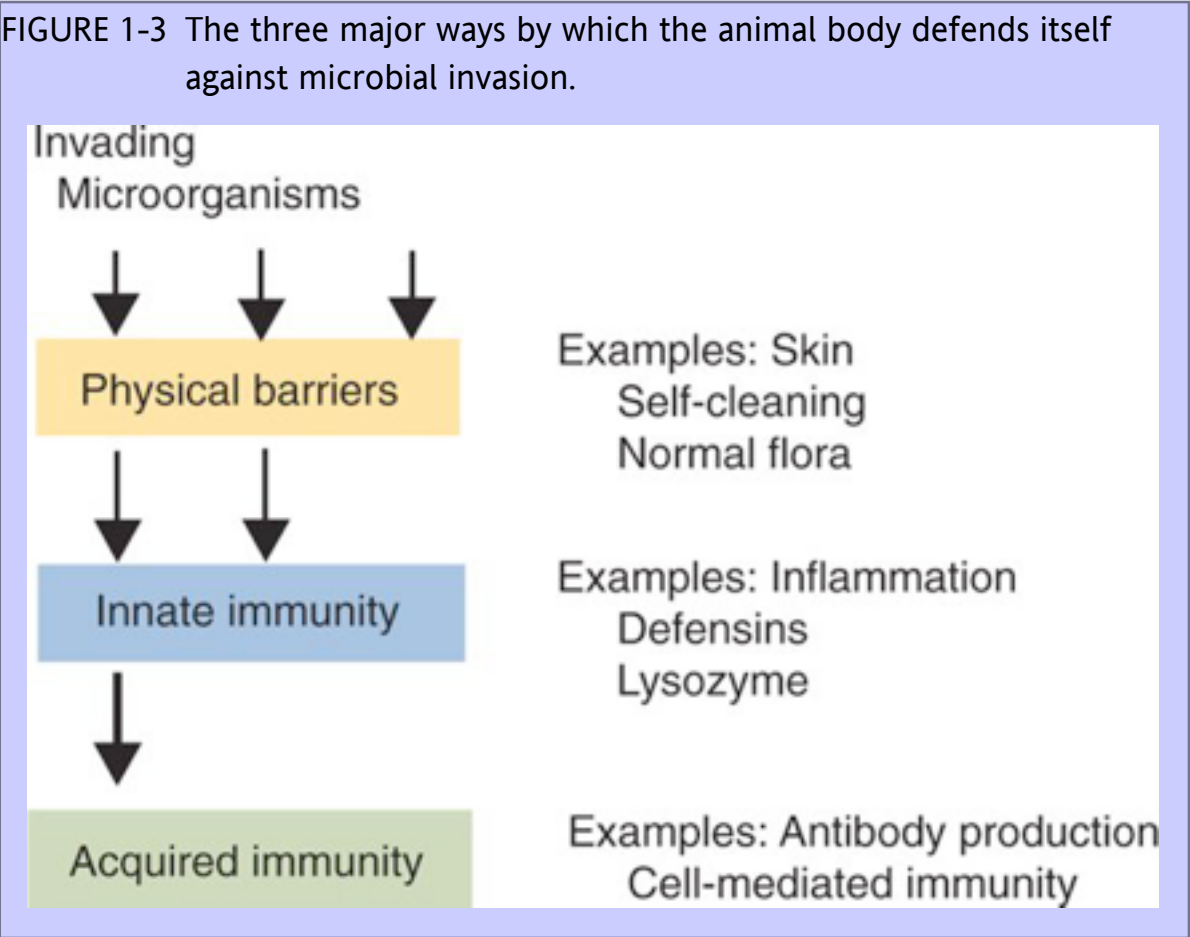


Table 1-1 A Comparison of Innate and Acquired Immunity

	Innate Immunity Always “on”	Acquired Immunity Turned on by Antigens
Cells engaged	Macrophages, dendritic cells, neutrophils, NK cells	T and B cells
Evolutionary history	Ancient	Recent
Onset	Rapid (min-hr)	Slow (days-weeks)
Specificity	Common microbial structures	Unique antigens
Potency	May be overwhelmed	Rarely overwhelmed
Memory	None	Significant memory
Effectiveness	Does not improve	Improves with exposure

The animal body can focus its innate defense mechanisms on sites of microbial invasion in the complex set of reactions we call inflammation. During inflammation, changes in tissues brought about by microbial invasion or

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tissue damage result in increased blood flow and the accumulation of cells that can attack and destroy the invaders. These cells, called neutrophils and macrophages, can destroy most invading organisms and so prevent their spread to uninfected areas of the body. The body also uses enzymes that are triggered by the presence of invaders to cause microbial destruction. These enzymes form what is known as the complement system. Some of the cells involved in inflammation may also repair damaged tissues once the invaders have been destroyed.

Animals also possess natural antimicrobial molecules such as the carbohydrate-digesting enzyme lysozyme and many carbohydrate-binding proteins. Some of these molecules circulate all the time; others are induced by the presence of bacteria or damaged tissues. These proteins can bind to invading organisms and accelerate their destruction.

The innate immune system lacks any form of memory, and each infection is treated identically. The intensity and duration of processes such as inflammation therefore remain unchanged no matter how often a specific invader is encountered. On the other hand, it is always ready to respond immediately once an invader is detected.

1.4.3

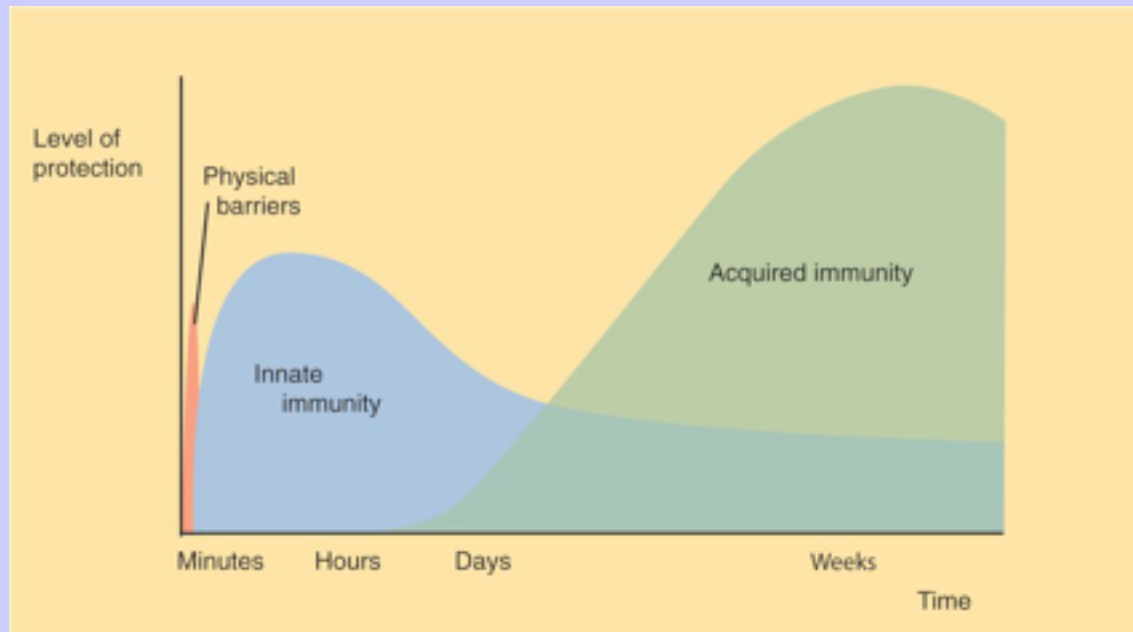
Acquired Immunity

Inflammation and the other components of the innate immune system are critical to the defense of the body. Animals that cannot mount an effective innate response will die from overwhelming infections. Nevertheless, these innate mechanisms cannot offer the ultimate solution to the defense of the body. What is really needed is a defense system that can recognize and destroy invaders and then learn from the process, so that if they invade again, they will be destroyed even more effectively. In this system, the more often an individual encounters an invader, the more effective will be its defenses against that organism. This type of adaptive response is the function of the acquired immune system.

The acquired immune system takes at least several days to become effective ([Figure 1-4](#)). Although it develops rather slowly, it is incredibly effective. When an animal eventually develops acquired immunity to an invader, the chances of successful invasion by that organism are reduced to a very low level. The animal may, in fact, be totally immune. The acquired immune system is a complex and sophisticated system that provides the ultimate defense of the body. Its importance is readily seen when it is destroyed. Thus in human AIDS patients, loss of acquired immunity leads inevitably to uncontrolled infections and death.

A key difference between the innate and acquired immune systems lies in their use of receptors to recognize foreign invaders. The innate system uses preexisting receptors that can bind to molecules and molecular patterns commonly found on many different microbes. In contrast, the cells of the acquired immune system randomly generate enormous numbers of structurally unique receptors. These receptors can bind to an enormous array of foreign molecules. Because the binding repertoire of these receptors is

FIGURE 1-4 The time course of innate and acquired immunity. Surface barriers provide immediate protection. Innate mechanisms provide rapid protection that keeps microbial invaders at bay until acquired immunity can develop. It may take several days or even weeks for acquired immunity to become effective.



generated randomly, they are not predestined to recognize any specific foreign molecule but collectively can recognize almost any invading microorganism.

The acquired immune system can recognize foreign invaders, destroy them, and retain the memory of the encounter. If the animal encounters the same organism a second time, the immune system responds more rapidly and more effectively. Such a sophisticated system must of necessity be complex. One reason for this complexity is the diversity of potential invaders. Microbial invaders fall into two broad categories. One category consists of the organisms that originate outside the body. These include most bacteria and fungi, as well as many protozoa and invading helminths. The second category consists of the organisms that originate or live inside the body's own cells. These include viruses and intracellular bacteria or protozoa. The acquired immune system therefore consists of two major branches that defend against each of these two categories of invaders. Thus one branch of the immune system is directed against the extracellular or exogenous invaders. Proteins called antibodies promote the destruction of these invaders. This type of immune response is sometimes called the humoral immune response since antibodies are found in body fluids (or "humors"). The other major branch of the immune system is directed against the intra-cellular or endogenous invaders that invade cells. Specialized cells destroy these infected or abnormal cells. This type of response is therefore called the cell-mediated immune response.

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1.5 ANTIBODY-MEDIATED IMMUNE RESPONSES

Soon after Pasteur discovered that it was possible to produce immunity to infectious agents by vaccination, it was recognized that the substances that provided this immunity could be found in the blood serum ([Figure 1-5](#)). For example, if serum is taken from a horse that has been vaccinated against tetanus (or has recovered from tetanus) and injected into a normal horse, the recipient animal will become resistant to tetanus for several weeks ([Figure 1-6](#)).

The protective molecules found in the serum of an immunized animal are proteins called antibodies. Antibodies against tetanus toxin are not found in the serum of normal horses but are produced following exposure to tetanus toxin as a result of infection or vaccination. Tetanus toxin is an example of a foreign substance that stimulates an immune response. The general term for such a substance is antigen. If an antigen is injected into an animal, then antibodies will be produced that can bind to that antigen and ensure its destruction. Antibodies are specific and only bind to the antigen that stimulates their production. For example, the antibodies produced in response to tetanus toxin bind only tetanus toxin. When the antibodies bind, they “neutralize” the toxin so that it is no longer toxic for animals. In this way antibodies protect animals against the lethal effects of tetanus.

The time course of the antibody response to tetanus toxin can be followed by taking blood samples from a horse at intervals after injection of the toxin (or injection of chemically detoxified toxin called tetanus toxoid, a much safer procedure). The blood is allowed to clot, and the clear serum is removed. The amount of antibody in the serum may be estimated by measuring its ability to neutralize a standard amount of toxin. Following a single injection of toxin into a horse that has never been previously exposed to it, no antibody is detectable for several days ([Figure 1-7](#)).

This lag period lasts for about one week. When antibodies eventually appear in serum, their level climbs to reach a peak by 10 to 20 days before declining and disappearing within a few weeks. During this first or primary response the amount of antibody formed, and there

FIGURE 1-5 The difference between serum and plasma. Plasma contains blood-clotting molecules that are absent from serum.

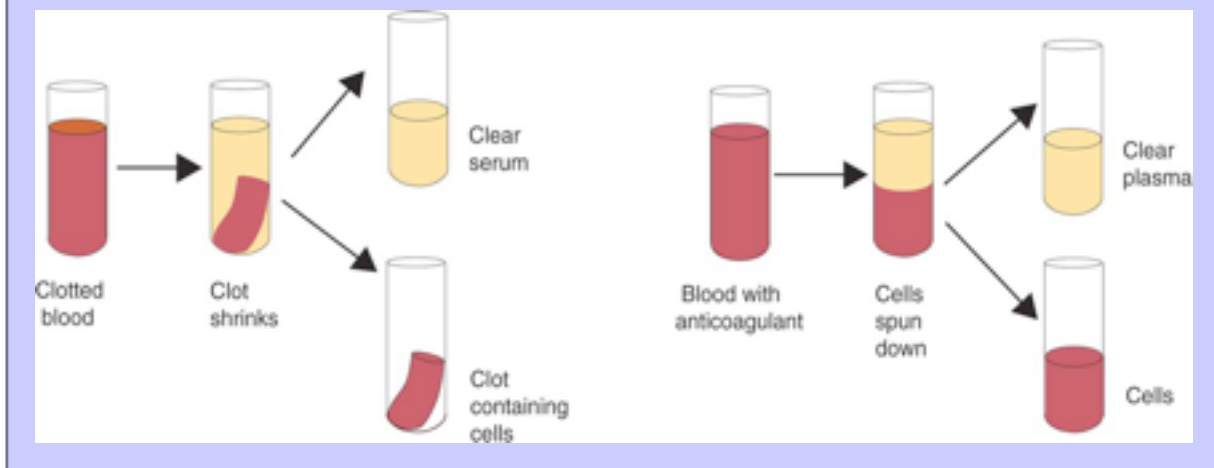


FIGURE 1-6 Transfer of immunity to tetanus by means of serum derived from an immunized horse. This clearly demonstrates that antibodies in serum are sufficient to confer immunity to tetanus.

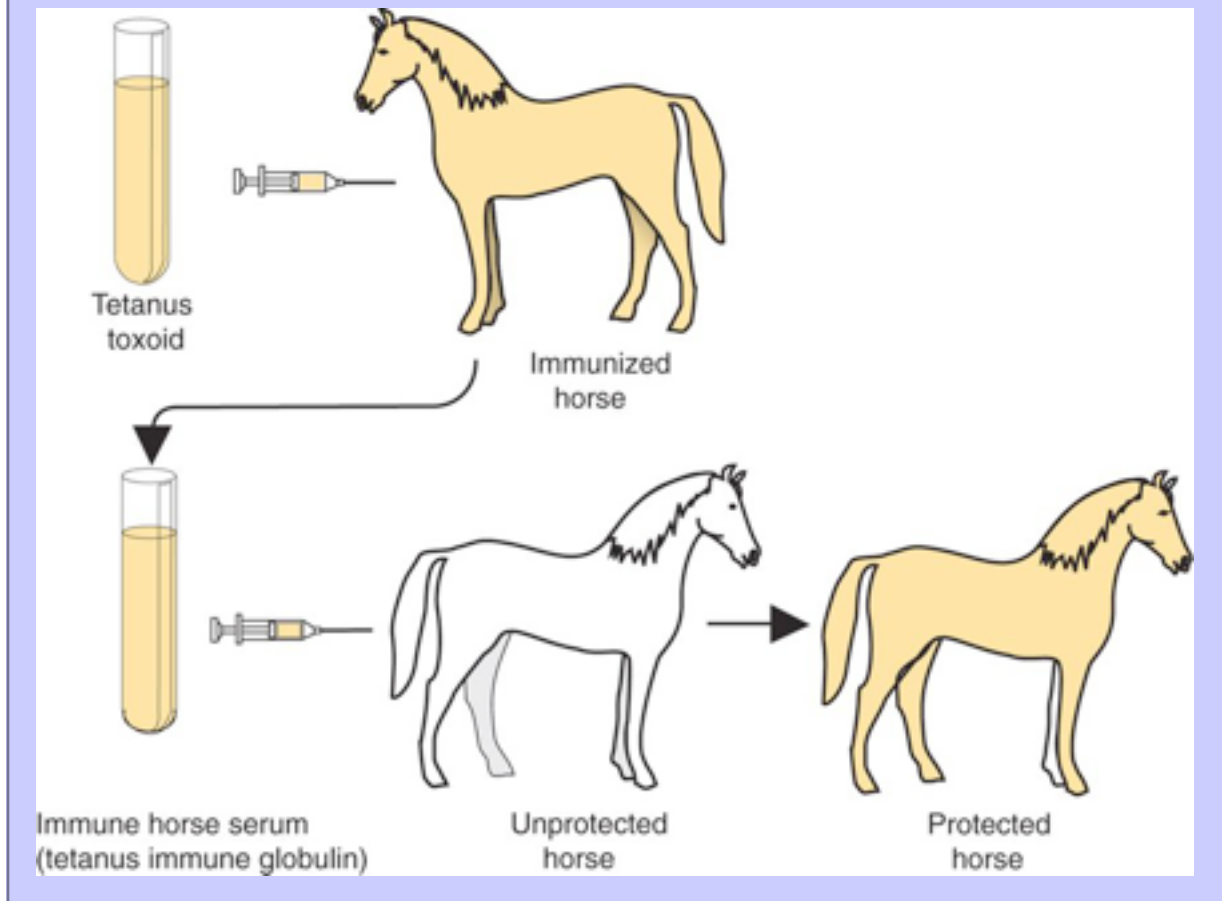
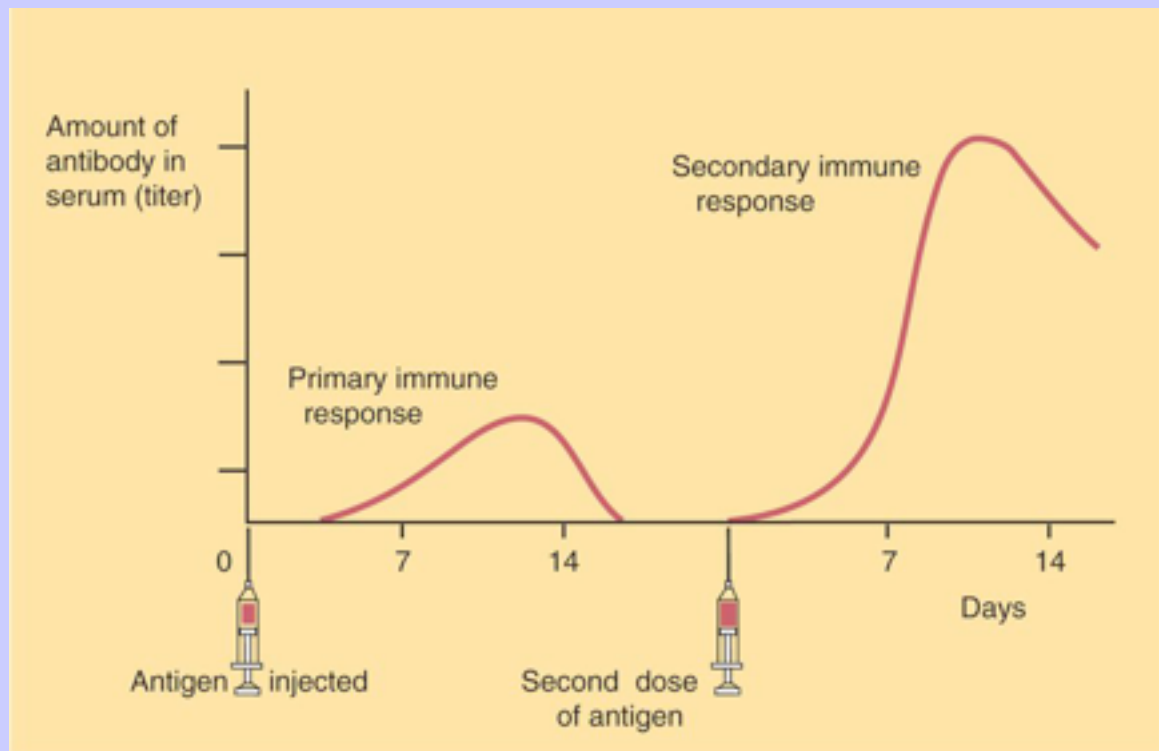


FIGURE 1-7 The characteristic time course of the immune response to an antigen as measured by serum antibody levels. Note the key differences between a primary and a secondary immune response.



fore the amount of protection conferred, is relatively small.

If, sometime later, a second dose of toxin or toxoid is injected into the same horse and the antibody response follows, the lag period lasts for no more than 2 or 3 days. The amount of antibody in serum then rises rapidly to a high level before declining slowly. Antibodies may be detected for many months or years after this injection. A third dose of the antigen given to the same animal results in an immune response characterized by an even shorter lag period and a still higher and more prolonged antibody response. As will be described later in this book, the antibodies produced after repeated injections are better able to bind and neutralize the toxin than those produced early in the immune response. The enhancement of the immune responses to infectious agents by repeated injections of antigen forms the basis of vaccination.

Compared to its response to the first dose, the animal's response to a second dose of antigen occurs much more quickly, antibodies reach much higher levels, and it lasts much longer. This secondary response is specific in that it can be provoked only by a second dose of an antigen. A secondary response may be provoked many months or years after the first injection of antigen, although its size tends to decline as time passes. A secondary response can also be induced, even though the response of the animal to the first injection of antigen was so weak as to be undetectable. These features of the secondary response indicate that the antibody-forming system possesses the ability to "remember" previous exposure to an antigen. For this reason, the secondary immune response is sometimes called an anamnestic response (*anamnesis* is Greek for "memory"). It should be noted, however, that repeated injections of antigen do not lead indefinitely to greater and greater immune responses. The level of antibodies in serum is

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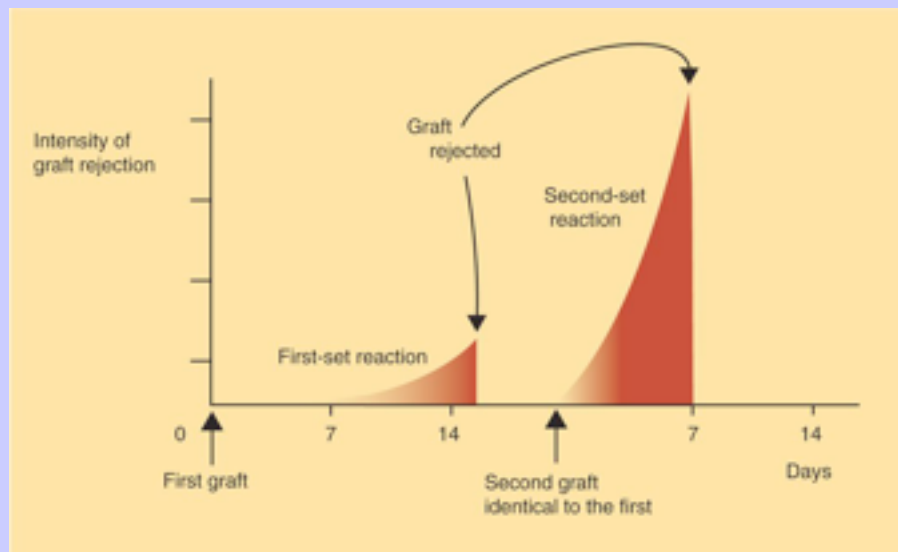
regulated so that it eventually stops rising, even after multiple doses of antigen or exposure to many different antigens.

1.6 CELL-MEDIATED IMMUNE RESPONSES

If a piece of living tissue such as a kidney or a piece of skin is surgically removed from one animal and grafted onto another of the same species, it usually survives for a few days before being rejected by the recipient. This process of graft rejection is significant because it demonstrates the existence of a mechanism whereby foreign cells, differing only slightly from an animal's own normal cells, are rapidly recognized and destroyed. Even cells with minor structural abnormalities may be recognized as foreign by the immune system and destroyed, even though they are otherwise apparently healthy. These abnormal cells include aged cells, virus-infected cells, and some cancer cells. The immune response to foreign cells as shown by graft rejection demonstrates that the immune system can identify and destroy abnormal cells.

If a piece of skin is transplanted from one dog to a second, unrelated dog, it will survive for about 10 days. The grafted skin will initially appear to be healthy, and blood vessels will develop between the graft and its host. By one week, however, these new blood vessels will begin to degenerate, the blood supply to the graft will be cut off, and the graft will eventually die and be shed ([Figure 1-8](#)). If a second graft is taken from the original donor and placed on the same recipient, then that second graft will survive for no more than a day or two before being rejected. Thus the rejection of a first graft is relatively weak and slow and analogous to the primary antibody response, whereas a second graft stimulates very rapid and powerful rejection similar in many ways to the secondary antibody response. Graft rejection, like antibody formation, is a specific immune response in that a rapid secondary reaction occurs only if the second graft is from the same donor as the first. Like antibody formation, the graft rejection process also involves memory, since a second graft may be rapidly rejected many months or years after loss of the first.

FIGURE 1-8 The characteristic time course of the rejection of a foreign skin graft. The intensity of the rejection process is much more severe when a secondary response is mounted. Notice how similar this diagram is to [Figure 1-7](#).



However, graft rejection is not entirely identical to antibody-mediated immunity because it cannot be transferred from a sensitized to a normal animal by means of serum. The ability to mount a secondary reaction to a graft can only be transferred between animals by living cells. The cells that do this are called lymphocytes and are found in the spleen, lymph nodes, or blood. The process of graft rejection is mediated primarily by lymphocytes and not by serum antibodies. It is a good example of a cell-mediated immune response.

1.7

MECHANISMS OF ACQUIRED IMMUNITY

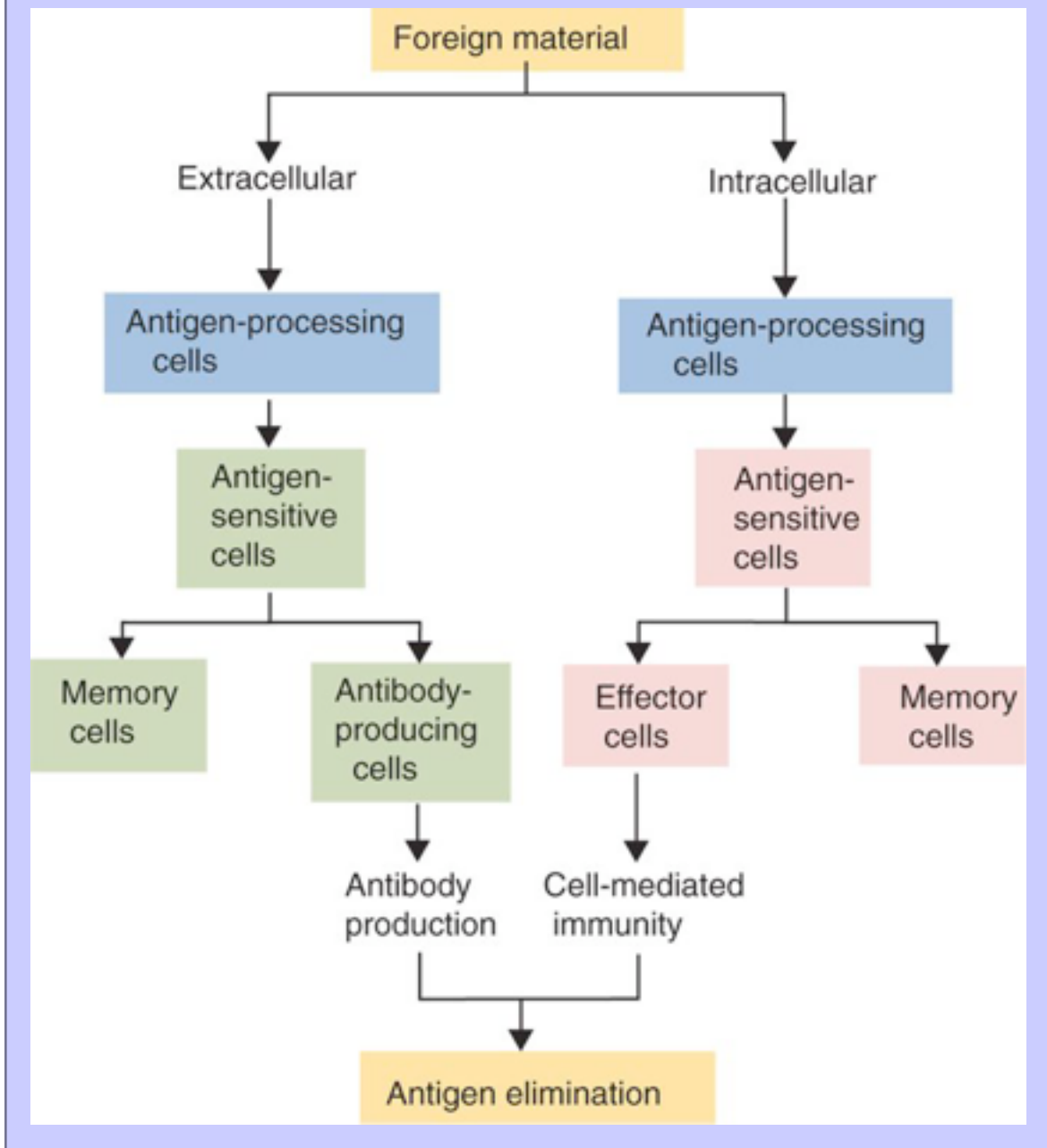
In some ways the acquired immune system may be compared to a totalitarian state in which foreigners are expelled, citizens who behave themselves are tolerated, but those who “deviate” are eliminated. While this analogy must not be carried too far, clearly such regimes possess a number of characteristic features. These include border defenses and a police force that keeps the population under surveillance and promptly eliminates dissidents. In the case of the acquired immune system, the antibody-mediated responses would be responsible for keeping the foreigners out whereas the cell-mediated responses would be responsible for stopping internal dissent. Organizations of this type also tend to develop a pass system, so that foreigners or dissidents not possessing certain identifying features are rapidly detected and dealt with.

Similarly, when a foreign antigen enters the body it first must be trapped and processed so that it can be recognized as being foreign. If so recognized, then this information must be conveyed either to the antibody-forming system or to the cell-mediated immune system. These systems must then respond by producing specific antibodies and/or cells capable of eliminating the antigen. The acquired immune system must also remember this event so that the next time an animal is exposed to the same antigen, its response will be faster and more efficient. The immune system also learns how to make antibodies or cells that can bind more strongly to the invader. In our totalitarian state analogy, the police force would be trained to recognize selected foreigners or dissidents and respond more promptly when they are encountered.

We can therefore observe that the acquired immune system includes five major components ([Figure 1-9](#)).

1. Cells that can trap and process antigen and then present it for recognition to the cells of the immune system.
2. Cells that have receptors for the processed antigen. These cells can bind and respond to the antigen (antigen-sensitive cells).

FIGURE 1-9 A simple flow diagram showing the essential features of the acquired immune responses.



3. Cells that, once activated by antigen, will produce specific antibodies or will participate in the cell-mediated immune responses against the antigen (effector cells).
4. Cells that will retain the memory of the event and react rapidly to that specific antigen if it is encountered at a later time.

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5. Cells that regulate this response and ensure that it functions at an appropriate level.

All of these cell populations can be recognized within the body. Antigen is trapped, processed, and presented by several cell types, including dendritic cells and macrophages. Lymphocytes called B and T cells have specific receptors for foreign antigen and are thus able to bind the processed antigen and respond appropriately.

Lymphocytes also function as memory cells and therefore initiate a secondary immune response. The lymphocytes that mediate the cell-mediated responses are T cells. The lymphocytes that mediate the antibody-mediated responses are B cells. The immune response is mainly regulated by populations of T cells. Those that promote immune responses are called helper T cells. Those that inhibit immune responses are called regulatory T cells.

In subsequent chapters we will first review the mechanisms involved in innate immunity. Following that, we will review acquired immunity in detail and examine each of its basic components in turn. We will then examine the role of the immune system in protecting animals against microbial invasion. We will also see what happens when the immune system functions abnormally, either excessively or inadequately.

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1.8 WHERE TO GO FOR ADDITIONAL INFORMATION

Many veterinary journals carry articles of interest to immunologists. Some of the most important are as follows: *Acta Veterinaria Scandinavica*, *American Journal of Veterinary Research*, *Australian Veterinary Journal*, *The Veterinary Journal*, *Canadian Journal of Comparative Medicine*, *Journal of the American Veterinary Medical Association*, *Journal of Comparative Pathology*, *Journal of Veterinary Internal Medicine*, *Research in Veterinary Science*, *Veterinary Immunology and Immunopathology*, *Veterinary Pathology*, and *The Veterinary Record*.

For information on new developments on basic immunology (with occasional papers on subjects of veterinary interest), the reader should review journals such as *Nature*, *Science*, *Journal of Immunology*, *Trends in Immunology*, *Proceedings of the National Academy of Sciences of the United States of America*, *New England Journal of Medicine*, *Infection and Immunity*, *Immunity*, *Immunogenetics*, and *Immunology*.

As in many scientific fields, the World Wide Web can be a very useful source of information on veterinary immunology, although care should be taken to verify the information provided. Some important sites include PubMed (<http://www.ncbi.nlm.nih.gov/sites/entrez>), which provides rapid access to scientific journals, and the Comparative Immunoglobulin Workshop (<http://www.medicine.uiowa.edu/cigw/>), which provides current information on immunoglobulin structures. Online resources for immunology research using animal models can be found at this site: http://www.animal.ufl.edu/hansen/Immunology_resources/VETIMMUNOLRESOURCES.htm. Readers may also wish to visit the website of the American Association of Veterinary Immunologists at <http://www.theaavi.org/> or national immunology organizations such as the American Association of Immunologists at www.aai.org/. The British Society for Immunology has a website showing a guide to the immune system at http://www.immunology.org/resources_gdoi.php.

² CHAPTER 2 How Inflammation Is Triggered

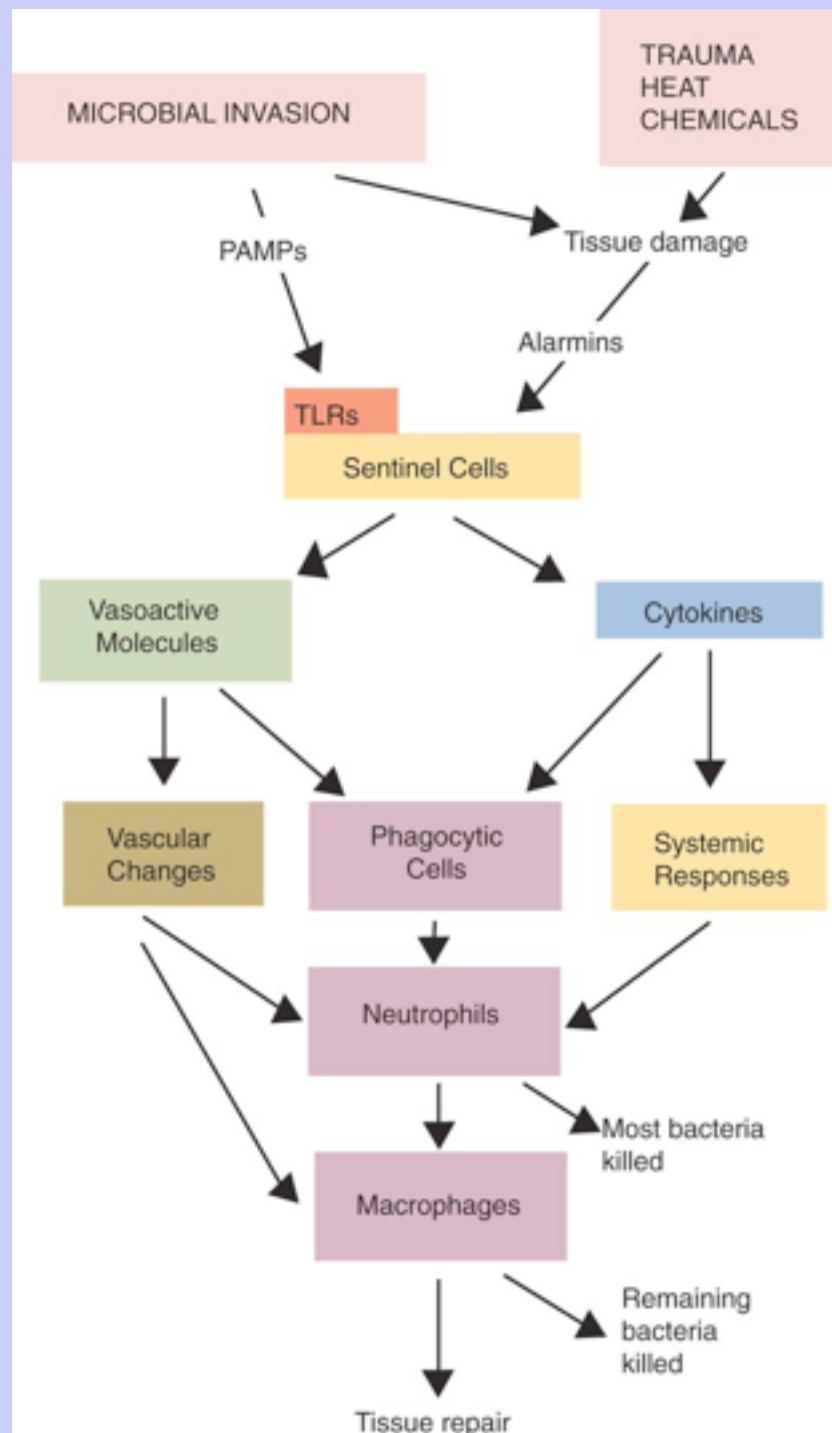
2.1 KEY POINTS

- The body recognizes invading microorganisms by the common molecules expressed on their surface, or by their characteristic nucleic acids. These are called pathogen-associated molecular patterns (PAMPs). It also recognizes the appearance of molecules released from damaged tissues. These are called alarmins.
- PAMPs are recognized by toll-like receptors (TLRs) on cell surfaces and by other receptors located within cells.
- Pattern-recognition receptors are found on many cell types. The most important cells are macrophages, dendritic cells (DCs), and mast cells, since these act as sentinels.
- Signals from TLRs cause the sentinel cells to be activated and to secrete many different molecules. Some of these molecules are cytokines that “turn on” the inflammatory process.
- The major proinflammatory cytokines are tumor necrosis factor- α (TNF- α), interleukin-1 (IL-1), and interleukin-6 (IL-6) as well as many different chemokines.
- Some of these molecules trigger local increases in blood flow and increased vascular permeability.

Infectious agents such as bacteria or viruses can grow very rapidly. A single bacterium with a doubling time of 50 minutes can produce about 500 million offspring within 24 hours. Thus if such a microorganism invades the body, it must be recognized and destroyed before it overwhelms the body. Time is of the essence, and delay can be fatal. The body must therefore employ fast-reacting preexisting immune mechanisms as its first line of defense against these invaders. The most important of these innate mechanisms is the process of acute inflammation.

Inflammation is vital because it ensures that defensive cells and molecules are concentrated rapidly at sites of microbial invasion and tissue damage. Inflammation involves the activation and directed migration of many different cells, especially neutrophils and macrophages, from the bloodstream to sites of invasion. Cells such as neutrophils are normally restricted to the bloodstream. They must migrate into infected tissues in order to destroy invaders. Likewise, many protective molecules, such as antibodies and complement components, are normally found only in blood.

FIGURE 2-1 An overview of the essential features of acute inflammation, an innate mechanism for focusing cells and other defensive mechanisms. It is triggered by microbial invasion and tissue damage.



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These large molecules enter the tissues only during inflammation. Inflammation thus provides a mechanism by which defenses are focused in a localized region ([Figure 2-1](#)). Inflammation permits cells and molecules to attack and destroy invaders. Later, when the invader is eliminated, the repair of damaged tissues can begin.

2.2 HOW INVADERS ARE RECOGNIZED

Inflammation is triggered when the body senses that it is under attack. This involves recognizing warning signals generated either by invading microorganisms or by dead and dying cells. There are two major groups of such warning signals. One group consists of molecules released from dead or dying cells. These are called alarmins. The other group consists of molecules or molecular patterns associated with microbial invaders. Collectively, these are called pathogen-associated molecular patterns (PAMPs). Together, the internally generated alarmins and the externally generated PAMPs form a family of damage-associated molecular patterns that can be recognized by cells dedicated to the body's defenses.

2.2.1 Pathogen-Associated Molecular Patterns

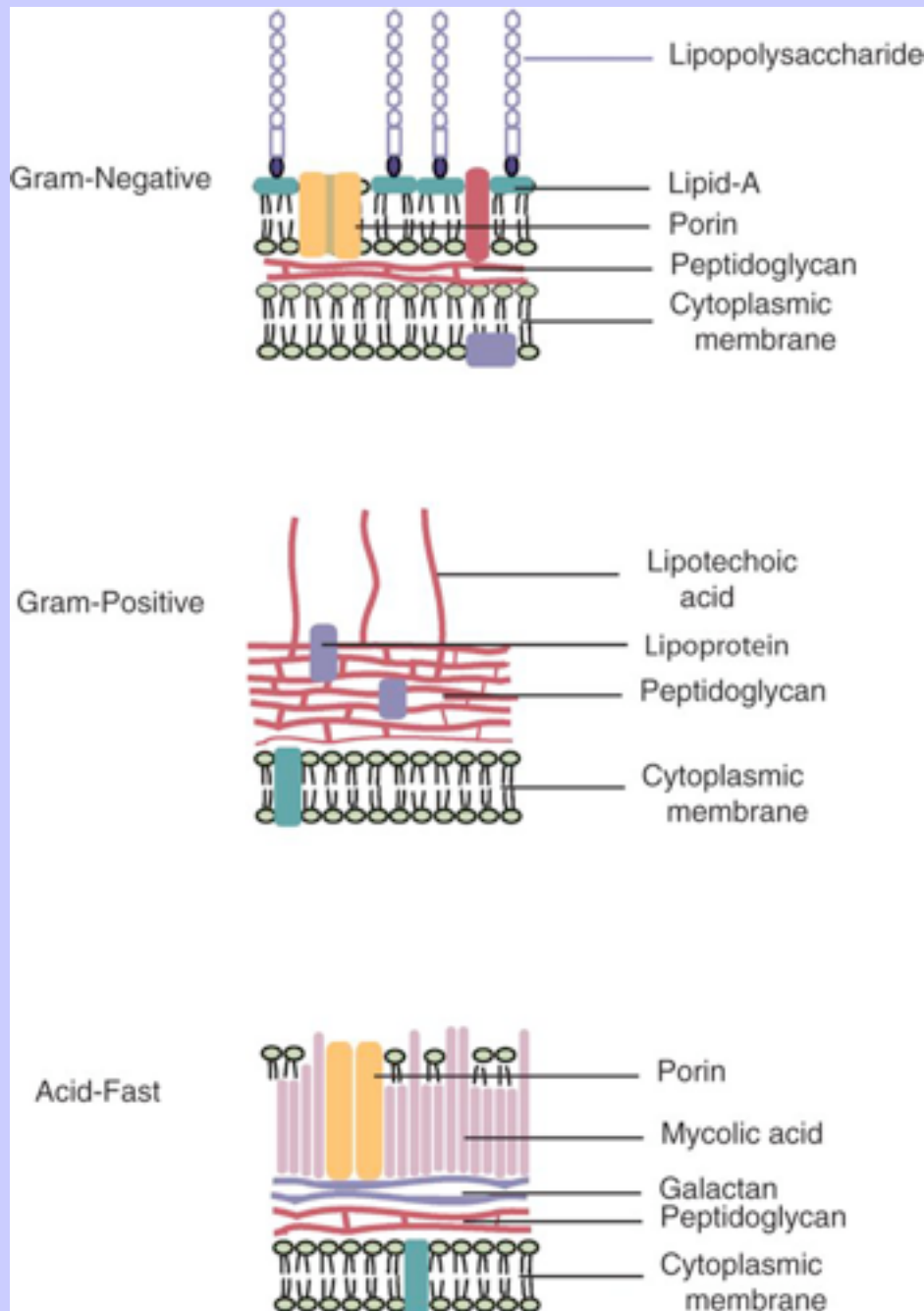
The presence of invading microbes is detected by “sentinel cells” such as macrophages, dendritic cells (DCs), and mast cells. These cells have receptors that can bind PAMPs expressed by bacteria, fungi, and viruses. Microbes not only grow fast, but also are highly diverse and can mutate and change their molecular structures much faster than any infected animal can respond. For this reason, sentinel cell receptors are not designed to recognize all possible microbial molecules. Rather, these cells use receptors that detect highly conserved molecules that are found in many different microorganisms. For example, most invasive bacteria are covered by a cell wall largely composed of complex carbohydrates. The walls of Gram-positive bacteria are largely composed of peptidoglycans (chains of alternating N-acetylglucosamine and N-acetylmuramic acid cross-linked by short peptide side chains) ([Figure 2-2](#)). Gram-positive bacterial cell walls also contain lipoteichoic acids. The cell walls of Gram-negative bacteria consist of peptidoglycans covered by a layer of lipopolysaccharide (LPS). Acid-fast bacteria are covered in glycolipids. Yeasts are also covered by a mannan-rich carbohydrate wall. None of these molecules is found in normal animal tissues. On the other hand, they are essential for microbial survival and are commonly shared by entire classes of pathogens. These PAMPs are therefore recognized by a set of “pattern-recognition receptors.” Many different pattern-recognition receptors have been identified, including receptors located on the cell surface and some located within the cytoplasm of the sentinel cells. Binding of PAMPs to these receptors activates intracellular signaling pathways and causes the sentinel cells to secrete molecules that trigger inflammation and other innate immune responses.

2.2.2 Toll-like Receptors

The most important of the pattern-recognition receptors are called toll-like receptors (TLRs). Some TLRs are located on cell surfaces, where they are well placed to encounter extracellular invaders. However, because

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FIGURE 2-2 The major structural features of the cell walls of Gram-negative, Gram-positive, and acid-fast bacteria. These conserved structural molecules serve as pathogen-associated molecular patterns and can bind to pattern-recognition receptors such as the toll-like receptors.



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viruses grow within cells, they must be detected by intracellular TLRs.

Table 2-1 PAMPs Recognized by the Mammalian Toll-like Receptors

TLR	Natural Ligands
TLR1	Diacylated lipoproteins
TLR2	Peptidoglycan, bacterial lipoproteins, zymosan, some LPS, spirochetes, mycobacteria, lipoteichoic acid, heat-shock protein, necrotic cells
TLR3	Double-stranded viral RNA
TLR4	LPS, lipoteichoic acid, viral protein, heat-shock protein, fibrinogen, saturated fatty acids, β -defensins, heparan sulfate
TLR5	Flagellin and flagellated bacteria
TLR6	Necrotic cells, diacylated lipoprotein, peptidoglycan (with TLR2)
TLR7	Single and double-stranded viral RNA
TLR8	Single-stranded viral RNA
TLR9	Unmethylated CpG bacterial DNA
TLR10	A pseudogene

TLRs are expressed by many different cell types. Most importantly, they are expressed on sentinel cells located on or near the surface of the body. These include macrophages, mast cells, and DCs, as well as eosinophils and the epithelial cells that line the respiratory and intestinal tracts. They owe their name to a closely related receptor called “Toll,” which was first identified in the fruit fly (*Drosophila*).

TLRs are single-chain, membrane glycoproteins. There are at least 14 different TLRs, numbered accordingly, and each serves as a receptor for one or more specific microbial molecules ([Table 2-1](#)). TLRs may either be expressed on cell surfaces (TLR2, 4, and 5) or within cells on endosomal membranes (TLR3, 7, and 9).

The cell-surface TLRs mainly recognize microbial proteins, lipoproteins, and LPS. The intracellular TLRs recognize viral nucleic acids. For example, TLR4 on the cell surface binds LPS from the surface of Gram-negative bacteria. TLR2, on the other hand, recognizes peptidoglycans, lipoproteins, and a glycolipid called lipoarabinomannan from *Mycobacterium tuberculosis*. TLR5 binds flagellin, the major protein of bacterial flagella. TLR9 is a cytoplasmic receptor for bacterial deoxyribonucleic acid (DNA). Bacteria must therefore be disrupted if this DNA is to be recognized. Both TLR3 and TLR7 bind viral double-stranded ribonucleic acid (RNA), whereas TLR 7 and TLR 8 are required for the recognition of viral single-stranded RNA. TLRs may also cooperate to bind PAMPs. For example, TLR2 can associate with TLR6, and the dual receptor complex can then recognize bacterial lipopeptides. Likewise, TLR1 associates with TLR2 to recognize mycobacterial lipoprotein. Given the number of possible TLR combinations, it is believed that the presently known TLRs can collectively recognize almost all infectious agents. TLR11 is somewhat different from the other TLRs. It is restricted to DCs, macrophages, and epithelial cells in the urinary tract, where it binds to bacteria and some PAMPs from protozoan parasites.

Once a cell-surface TLR binds a microbial PAMP (its ligand), a signal is passed to the cell. This results in an increase in a transcription factor called nuclear factor kappa-B (NF- κ B) ([Figure 2-3](#)). NF- κ B in turn activates the

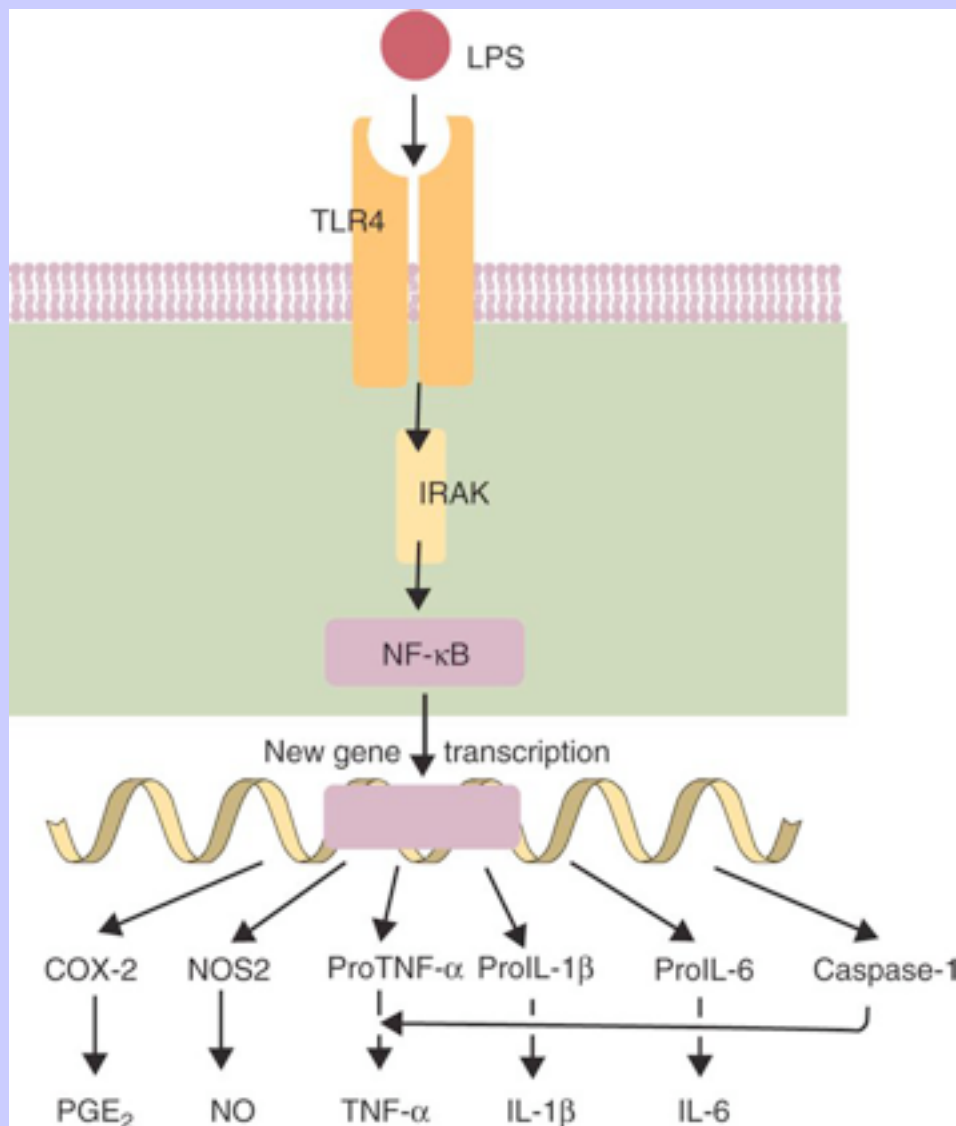
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genes that encode the cytokines, interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF- α). (For additional details see [Chapter 6](#).) Cytokines are proteins that regulate the activities

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FIGURE 2-3 Binding of a pathogen-associated molecular pattern such as lipopolysaccharide to a toll-like receptor (TLR) leads to generation of a transcription factor called nuclear factor kappa-B (NF- κ B). NF- κ B turns on the genes for three major cytokines, interleukin-1 (IL-1), IL-6, and tumor necrosis factor- α (TNF- α). It also turns on the genes for nitric oxide synthase 2 (NOS2) and cyclooxygenase-2 (COX-2). These two enzymes generate nitric oxide and prostaglandins and leukotrienes, respectively.



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of cells involved in the defense of the body. The cytokines are first made as pro-molecules that have to be activated by an enzyme called caspase-1. The production of caspase-1 is stimulated by a protein complex called an inflammasome that forms when microbial molecules bind to TLRs.

Table 2-2 Other Mammalian Pattern-Recognition Receptors

Receptor	Natural Ligands
Mannose-fucose receptor	Terminal mannose/fucose on microbial glycoproteins and glycolipids
CD14	Bacterial lipopolysaccharides
NOD1	Bacterial peptidoglycans
NOD2	Muramyl dipeptide
Peptidoglycan-recognition proteins	Bacterial peptidoglycans
RIG-like receptors	Viral RNA
CD1	Bacterial glycolipids
CD36	Bacterial lipoproteins
CD48	Fimbrial proteins

Caspases are a family of proteolytic enzymes, the cysteinyl-aspartate specific proteinases, that play key roles in the initiation of inflammation. Some of these caspases, such as caspase-1, 4, 5, and 12, are activated by signals generated by TLRs. Caspase-1 is the most important in this respect. It acts on the inactive precursors of IL-1, IL-6, and TNF- α to produce the active cytokines. These cytokines trigger the next phase of the inflammatory response.

Different TLRs trigger the production of different cytokine mixtures, and different PAMPs trigger distinctly different responses even within one cell type. For example, TLRs that recognize bacterial molecules tend to trigger the production of cytokines optimized to combat bacteria; those that recognize viral molecules will produce antiviral cytokines, and so forth.

TLRs not only trigger the innate immune defenses such as inflammation but also begin the process of “turning on” the acquired immune system. For example, stimulation of TLR4 makes macrophages and their close relatives, the DCs, produce cytokines that are potent stimulators of immune cells (see [Chapter 8](#)).

TLRs are expressed on hematopoietic stem cells—the bone marrow cells that produce leukocytes. Bacterial LPS binding to TLR4 stimulate the differentiation of these stem cells into leukocyte progenitors and cause the bone marrow to increase leukocyte production. An increase in leukocyte numbers in the blood (the white cell count) is a consistent feature of infectious diseases. This pathway also stimulates the development of DCs from lymphoid progenitors and so activates and replenishes the innate immune system during infections.

2.2.3

NOD-like Receptors

Nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) are a family of pattern-recognition receptors found inside cells. Unlike TLRs, which mainly detect extracellular microbes, NLRs can detect pathogens within the cytosol and when activated induce host defense signaling pathways ([Table 2-2](#)). Although TLRs and NLRs differ in their location and function, they share similar structures for microbial sensing and

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cooperate to initiate host responses to pathogens. NOD1 recognizes bacterial peptidoglycans. NOD2 recognizes muramyl dipeptide and serves as a general sensor of intracellular bacteria. Both NOD proteins act to generate NF- κ B.

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2.2.4

Peptidoglycan-Recognition Proteins

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Peptidoglycans are polymers of alternating N-acetyl glucosamine and N-acetyl muraminic acid found on both Gram-positive and Gram-negative bacteria. Peptidoglycan-recognition proteins (PGRPs) bind these microbial peptidoglycans and induce the production of antimicrobial peptides such as defensins. Although first identified in arthropods, they have since been found in humans, mice, cattle, and pigs. In pigs they are expressed constitutively in the skin, bone marrow, intestine, liver, kidney, and spleen. Their expression in intestinal tissues is increased by Salmonella infection. One member of this family, bovine PGRP-S, can kill microorganisms in which the peptidoglycan is either buried (Gram-negative bacteria) or absent (*Cryptococcus*), thus raising questions about its precise ligand. PGRP-S also binds bacterial LPS and lipotechoic acids. It is localized on the large granules of naïve neutrophils, which release PGRP-S when exposed to bacteria. Thus PGRP-S probably plays a significant role in the resistance of cattle to bacterial infections.

2.2.5

RIG-like Receptors

Retinoic acid inducible gene (RIG)-like receptors (RLRs) are pattern-recognition receptors expressed in the cytosol of cells, where they bind viral RNA. Viral RNA is different in several respects from mammalian RNA and so can be detected by these molecules. On interacting with viral RNA, RLRs initiate a cellular antiviral response and the production of antiviral cytokines called interferons.

2.2.6

Other Pattern-Recognition Receptors

The sentinel cells—macrophages, mast cells, and DCs—have many other receptors that can recognize microbial molecules. These include mannan receptors that bind microbial carbohydrates; scavenger receptors such as CD36 that can bind bacterial lipoproteins, and CD1, which binds microbial glycolipids.

2.2.7

Bacterial DNA

Bacterial DNA stimulates innate immunity. It differs from eukaryotic DNA in that it contains a large proportion of the dinucleotide cytosine-guanosine (CpG). In addition, while the cytosine in eukaryotic DNA is normally methylated, this is not the case in bacterial DNA. Thus unmethylated CpG dinucleotides are sufficiently different that they can bind TLR9 and so trigger innate immune responses. Bacterial DNA also contains deoxyguanosine (dG) nucleotides. These dG nucleotides form structures other than the usual double helix. One such structure is called quadriplex DNA. This binds to TLR9 and stimulates production of the cytokines IL-12, TNF- α , and IL-6.

2.2.8

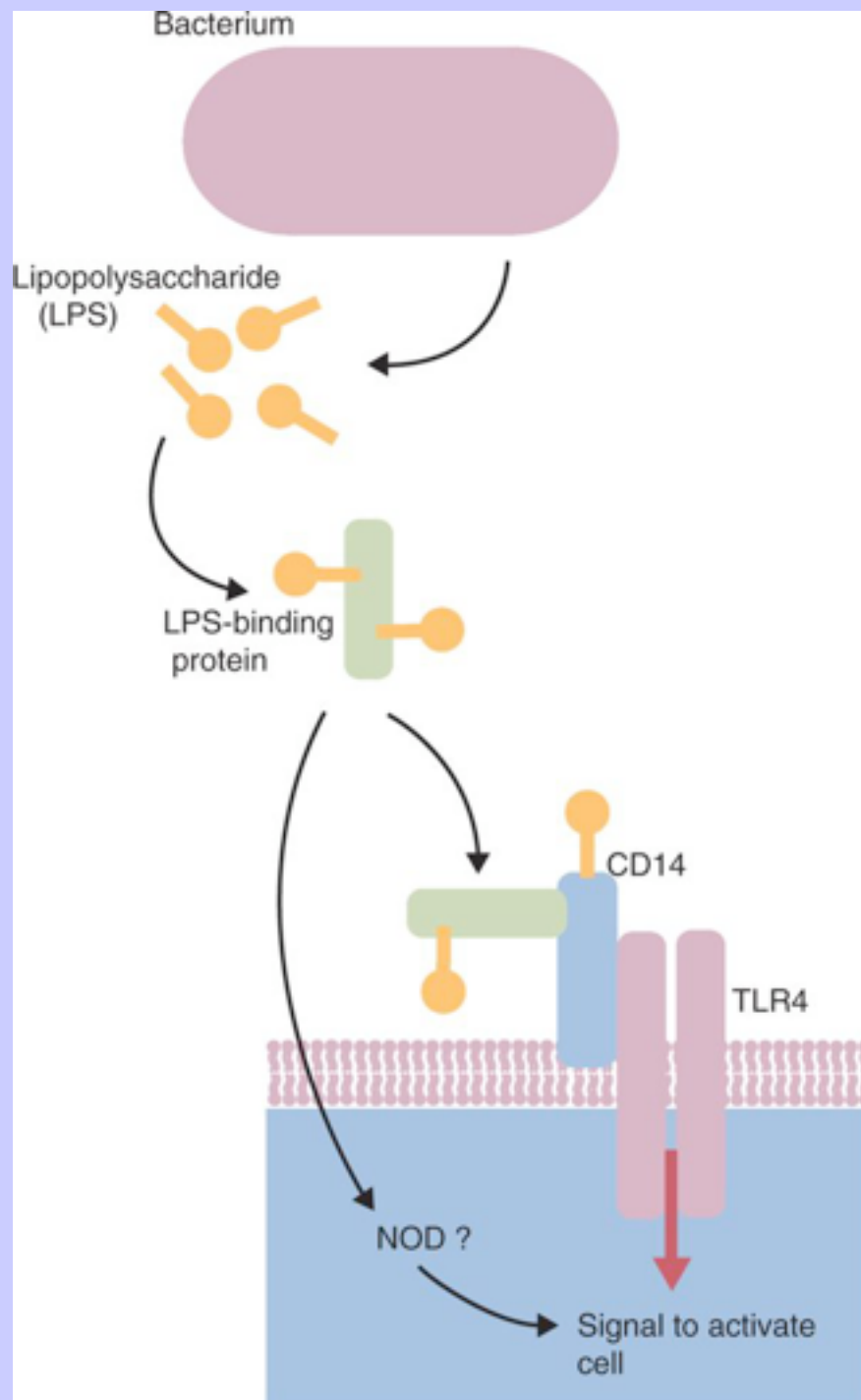
Bacterial Lipopolysaccharides

Bacterial LPS are potent inducers of innate immunity. They are released by invading Gram-negative bacteria. They do not act directly on cells but first bind to LPS-binding protein (LBP) in serum ([Figure 2-4](#)). LBP immediately transfers LPS molecules to a protein called CD14 located on the surface of macrophages ([Box 2-1](#)). CD14 cannot penetrate cell membranes and so is unable to signal to cells directly. CD14 therefore binds to TLR4 on the cell surface. Binding of LPS to the CD14/TLR4 complex activates macrophages and triggers cytokine

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production. The LPS subsequently dissociates from CD14 and binds to lipoproteins, where its toxic activities are lost. CD14 also binds many other microbial molecules: lipoarabinomannans from mycobacteria, manuronic acid polymers from *Pseudomonas*, and peptidoglycans from *Staphylococcus aureus*.

FIGURE 2-4 The processing and fate of bacterial lipo-polysaccharide.



2.2.8.1 Box 2-1 The CD System

When advances in immunology made it possible to make highly specific antibodies against individual cell surface proteins (see [Chapter 13](#)), it was soon shown that mammalian cells possessed hundreds of different surface proteins. Initially, each protein was given a specific name and often an acronym as well. It soon became clear, however, that such a system was unworkable. In an attempt to classify these proteins, a system has been established that assigns each protein to a numbered cluster of differentiation (CD). In many cases, a defined CD denotes a protein of specific function. For example, the protein CD14 binds bacterial lipopolysaccharide. As of May 2006, numbers up to CD350 have been assigned. Unfortunately, CD numbers provide no clue as to a molecule's function. As a result, in practice immunologists tend to use a mixed system using both a CD number and an abbreviation that denotes the function of a molecule. For example, CD32 is also called FcγR1. A list of selected CD molecules can be found in [Appendix 1](#).

2.2.9 The Complement System

Perhaps the most important of the innate protective systems that can destroy invading microbes is the complement system. This system consists of a large number of proteins found in the bloodstream. When exposed to invading bacteria, the complement system is activated through reactions involving several distinct pathways. For example, it can be triggered simply by exposure of complement proteins to microbial cell walls. This method of activation is called the alter-native complement pathway. Another complement pathway is activated when mannose-binding lectin encounters microbial cell walls. Once activated by either pathway, activated complement components can either kill microbes directly or prepare them for capture by phagocytic (eating) cells. The complement system is described in detail in [Chapter 5](#).

2.3 ALARMINs

The innate immune system must recognize not only PAMPs derived from invading microorganisms but also molecules released by damaged tissues. These molecules, collectively called alarmins, may be released when cells die. Alternatively, they may be secreted by stimulated sentinel cells. Alarmins are multifunctional and many have potent antimicrobial properties. They may recruit and activate cells of the innate immune system and indirectly promote acquired immune responses. Many different molecules can act as alarmins. They include defensins, cathelicidins, eosinophil-derived neurotoxin, and high mobility group box protein-1 (HMGB1). Other molecules that may be classified as alarmins include some chemokines, cytokines such as interleukin-1α (IL-1α), galectin-1, and S100 proteins (a family of calcium-binding proteins involved in cell growth and tissue injury). All are released in response to tissue damage and play key roles in innate immunity and tissue repair.

An example of an alarmin is heparan sulfate generated by broken cells. This molecule is normally restricted to cell membranes and the extracellular matrix but is shed into tissue fluids following injury. Heparan sulfate binds to TLR4 and so activates sentinel cells. Fibrinogen, a clotting protein, also stimulates macrophages through TLR4. Other alarmins include heat-shock proteins synthesized by cells under stress. These proteins bind TLR2 and TLR4.

2.3.1 HMGB1

HMGB1 was first described as a histone, a protein that binds DNA and ensures the correct folding of DNA molecules within the nucleus. It is found in all vertebrate cells and is highly conserved between species. However, HMGB1 has a second function. It is a cytokine with the ability to trigger inflammation. Thus HMGB1 is secreted

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by macrophages activated by LPS or proinflammatory cytokines such as interferon- γ . HMGB1 also leaks from necrotic or damaged and dying cells. Apoptotic cells, in contrast, do not release HMGB1 since these cells retain their nuclear integrity. HMGB1 binds to TLR2 and TLR4 and so stimulates cytokine production. HMGB1 sustains and prolongs inflammation since it induces the secretion of proinflammatory cytokines from macrophages, monocytes, neutrophils, and endothelial cells. Administration of HMGB1 to normal animals causes fever, weight loss, anorexia, acute lung injury, arthritis, and death. It plays a role in tissue repair since it stimulates the growth of new blood vessels. HMGB1 has potent antimicrobial activity. DCs can also secrete HMGB1 and this in turn promotes the proliferation and Th1 polarization of interacting T cells (see [Chapter 12](#)).

2.4 SENTINEL CELLS

The major sentinel cells—macrophages, DCs, and mast cells—are scattered throughout the body but are found in highest numbers just below body surfaces at locations where invading microorganisms are likely to be encountered. All can detect and then respond rapidly to PAMPs and alarmins.

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2.4.1 Macrophages

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Macrophages not only act as sentinel cells by detecting invading microorganisms, they can also kill invaders and play an essential role in triggering acquired immunity. When stimulated, they secrete cytokines that promote both innate and acquired immune responses; they control inflammation; and they contribute directly to the repair of damaged tissues by removing dead, dying, and damaged cells and so assist the healing process. Their name is derived from the fact that they are “large-eating” cells (Greek *macro*, *phage*).

Immature macrophages circulate in the blood, where they are called monocytes. When monocytes mature they migrate into tissues and become macrophages. Mature macrophages found in connective tissue are called histiocytes; those lining the sinusoids of the liver are called Kupffer cells; those in the brain are microglia. The macrophages found in the alveoli of the lungs are called alveolar macrophages, whereas those in the capillaries of the lung are called pulmonary intravascular macrophages. Large numbers are found in the sinusoids of the spleen, bone marrow, and lymph nodes. Irrespective of their name or location, they are all macrophages and all are part of the mononuclear phagocyte system ([Figure 2-5](#)).

2.4.1.1 Structure

Macrophages change their shapes in response to their environment. In suspension, however, they are round cells about 15 μm in diameter. They possess abundant cytoplasm, at the center of which is a single nucleus that may be round, bean shaped, or indented ([Figure 2-6](#)). Their central cytoplasm contains mitochondria, large numbers of lysosomes, some rough endoplasmic reticulum, and a Golgi apparatus, indicating that they can synthesize and secrete proteins ([Figures 2-7](#) and [2-8](#)). In living cells, the peripheral cytoplasm is in continuous movement, forming and reforming veil-like ruffles. Some macrophages show variations from this basic structure. Peripheral blood monocytes have round nuclei, which elongate as the cells mature. Alveolar macrophages rarely possess rough endoplasmic reticulum, but their cytoplasm is full of granules. The

FIGURE 2-5 The location of the cells of the mononuclear phagocyte system.

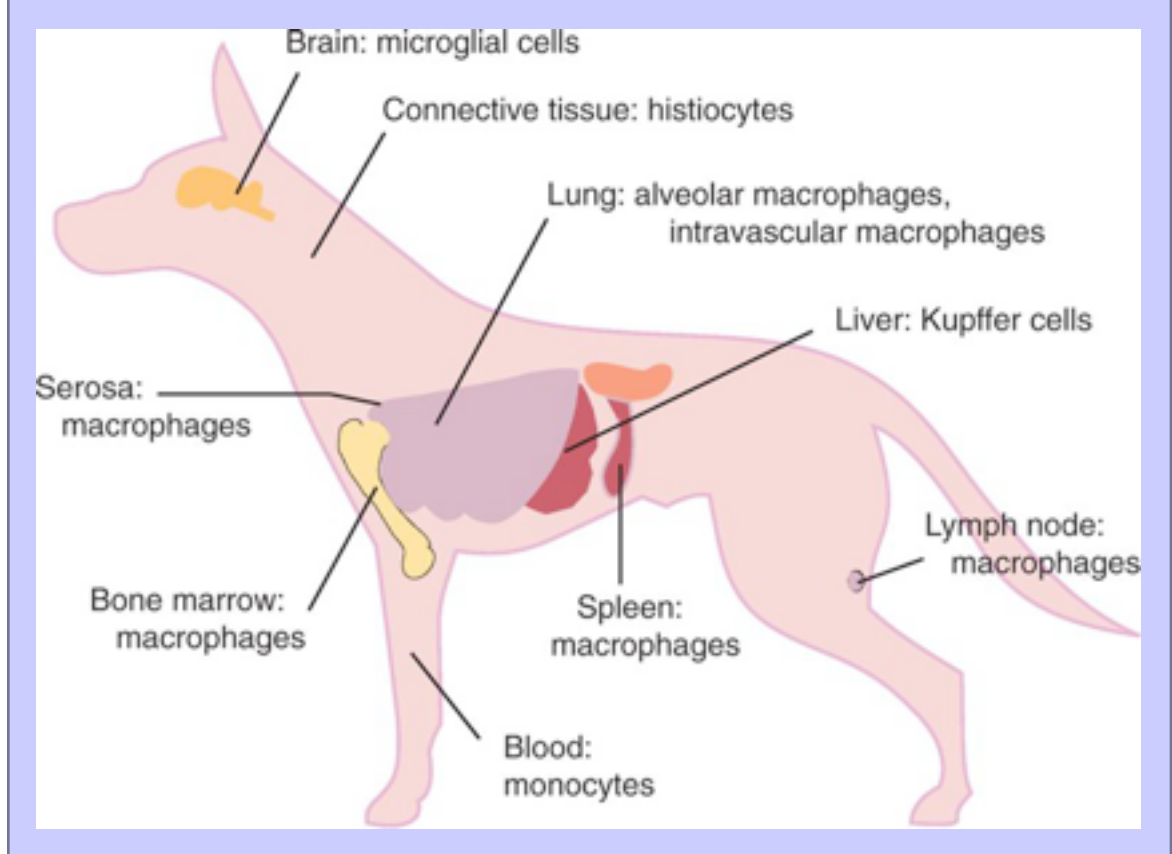
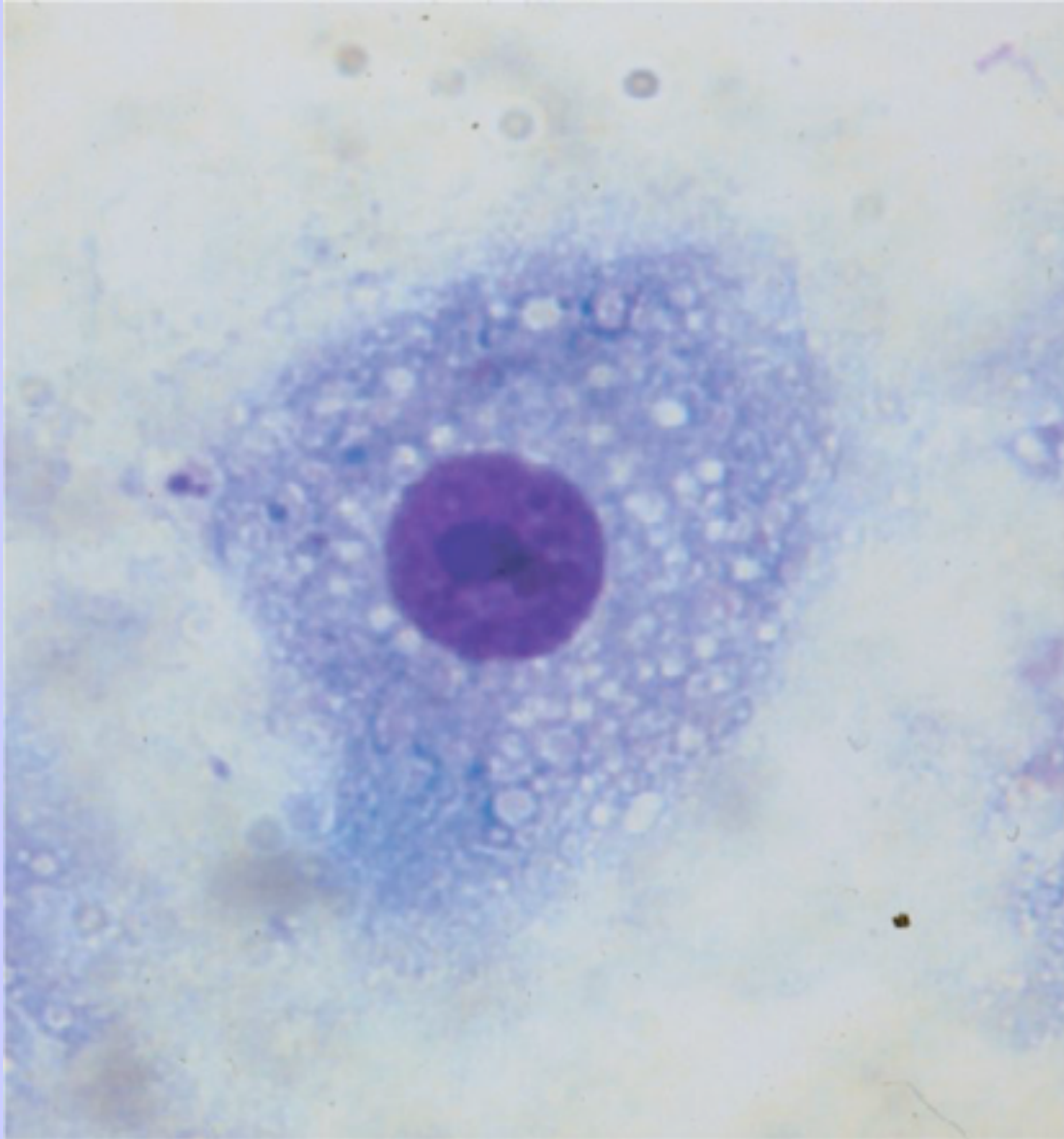


FIGURE 2-6 A typical macrophage, original magnification $\times 500$.



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FIGURE 2-7 The major structural features of a macro-phage.

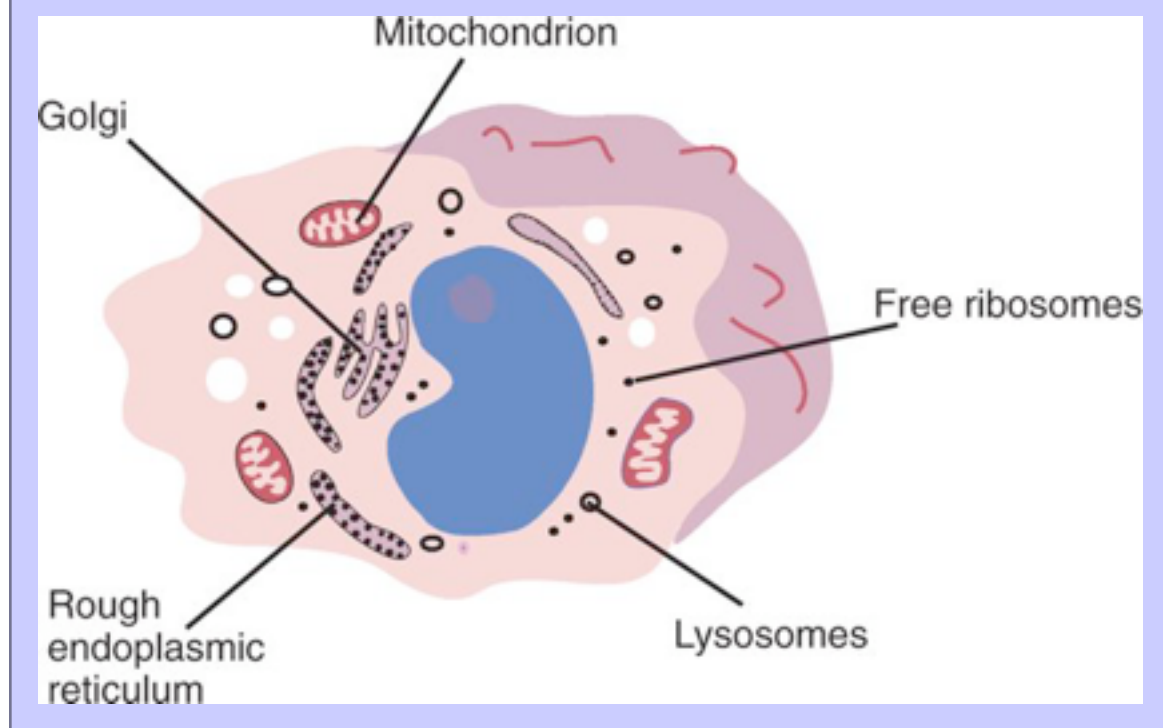


FIGURE 2-8 Transmission electron micrograph of a normal rabbit macrophage. The nature of the large inclusion is unknown. (Courtesy Dr. S. Linthicum.)

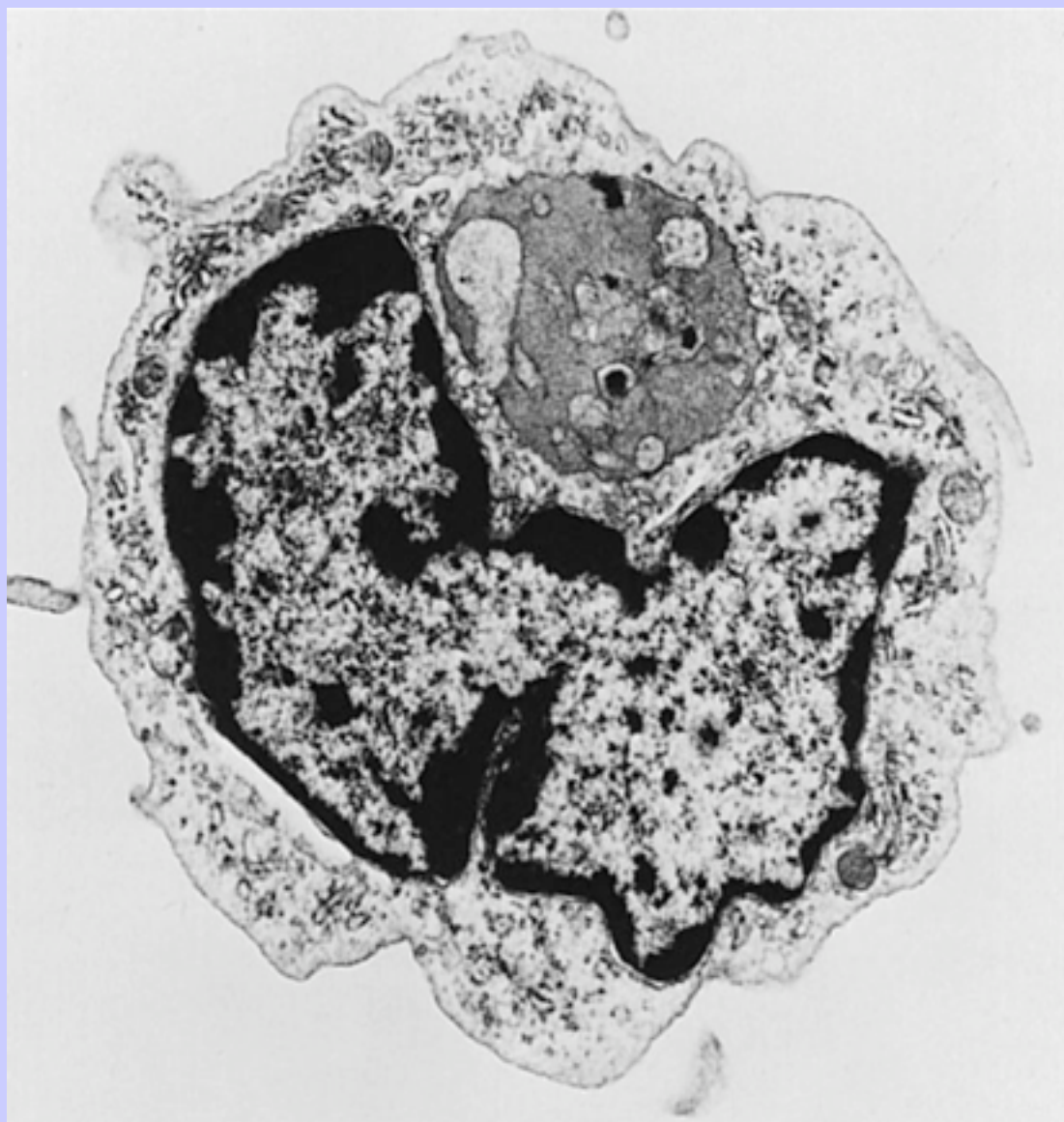
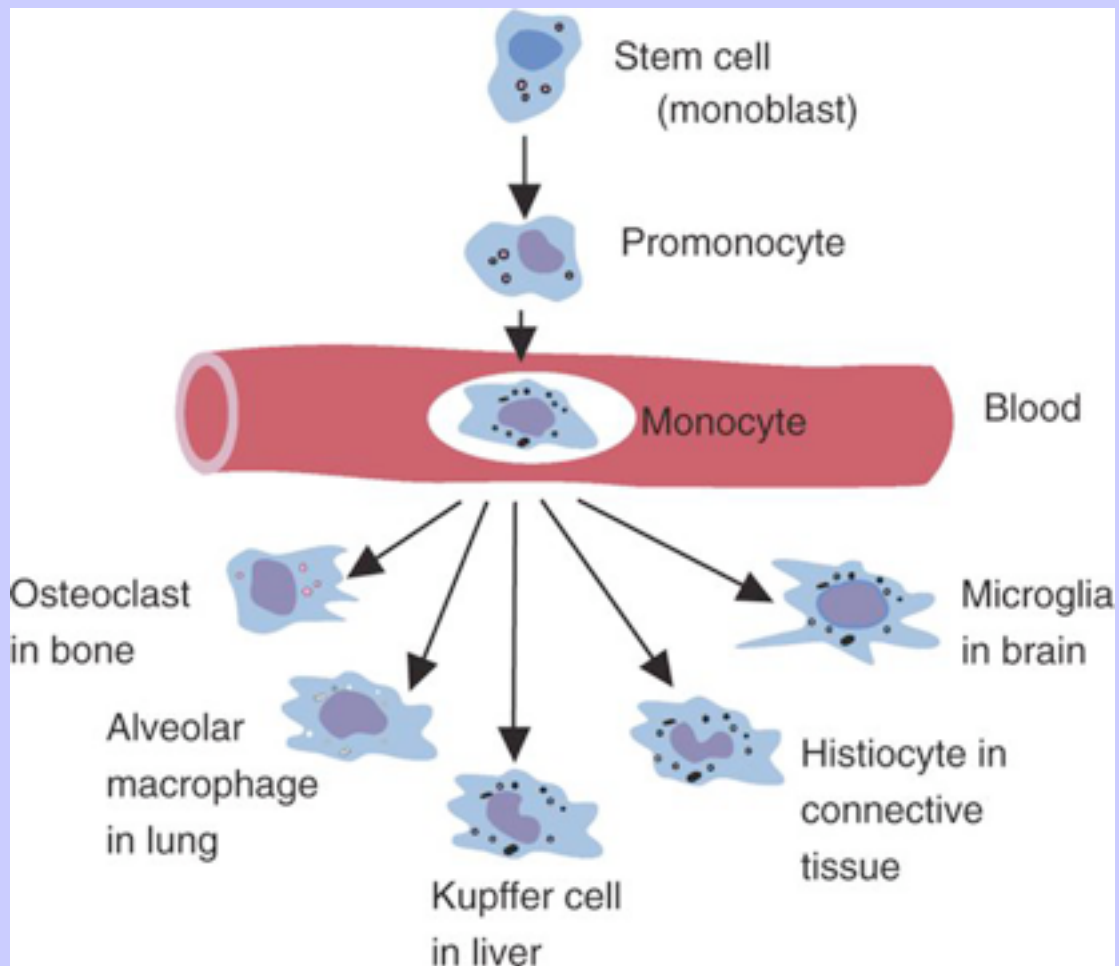


FIGURE 2-9 Origin and development of macrophages. Monocytes in blood can differentiate into many different types of macrophage.



microglia of the central nervous system have rod-shaped nuclei and very long cytoplasmic processes (dendrites) that are lost when the cell is stimulated by tissue damage.

2.4.1.2

Life History

All the cells of the mononuclear phagocyte system arise from stem cells in the bone marrow called monoblasts ([Figure 2-9](#)). Monoblasts develop into promonocytes, and promonocytes develop into monocytes, all under the influence of cytokines called colony-stimulating factors. Monocytes then enter the blood and circulate for about 3 days before entering tissues and developing into macrophages. They form about 5% of the total leukocyte population in blood. Tissue macrophages either originate from monocytes or divide within tissues. They are relatively long-lived cells, replacing themselves at a rate of about 1% per day unless activated by inflammation or tissue damage. Macrophages may live for a long time after ingesting chemically inert particles, such as the carbon injected in tattoo marks, although they may fuse together to form multinucleated giant cells in their attempts to eliminate the foreign material.

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2.4.2 Dendritic Cells

The second population of sentinel cells consists of DCs—so called because many possess long, thin cytoplasmic processes called dendrites. DCs are a very heterogeneous population of cells but many are closely related to macrophages. They are discussed in detail in [Chapter 8](#).

2.4.3 Mast Cells

2.4.3.1 Structure

Mast cells are large, round cells (15 to 20 μm in diameter) scattered throughout the body in connective tissue, under mucosal surfaces, in the skin, and around nerves ([Figure 2-10](#)). They are found in highest numbers at sites in the body exposed to potential invaders such as under the skin or in the intestine and airways. In these locations they are located close to

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FIGURE 2-10 A section of canine skin stained to show mast cells. The mast cells stain intensely because of the heparin in their cytoplasmic granules.

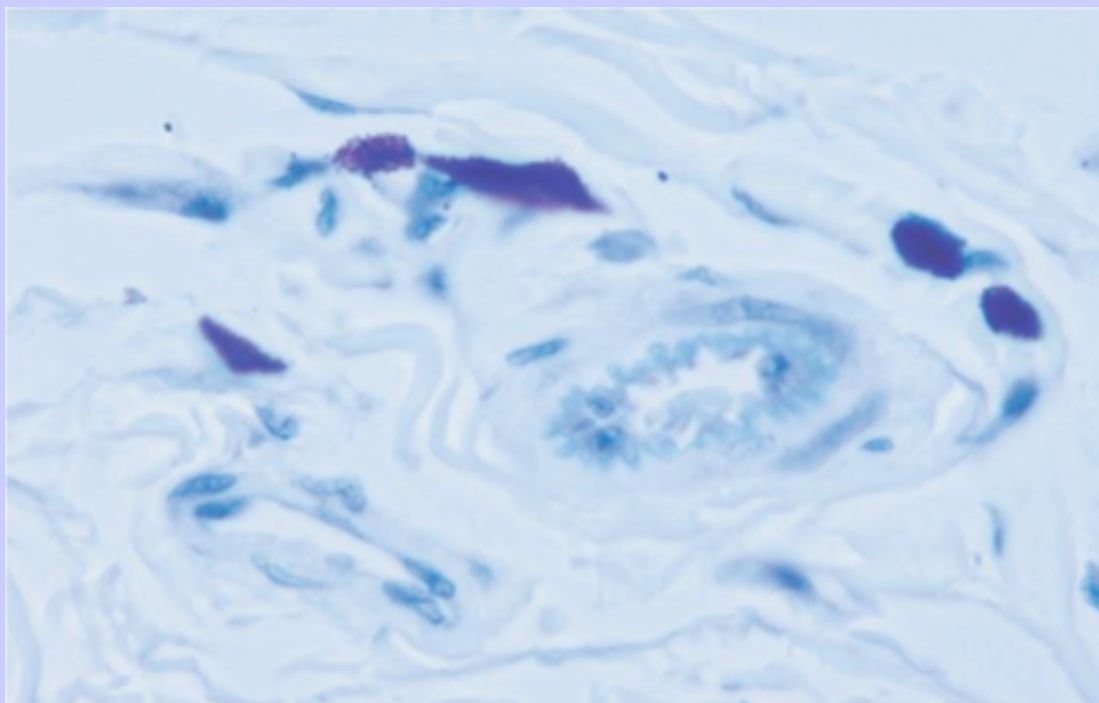
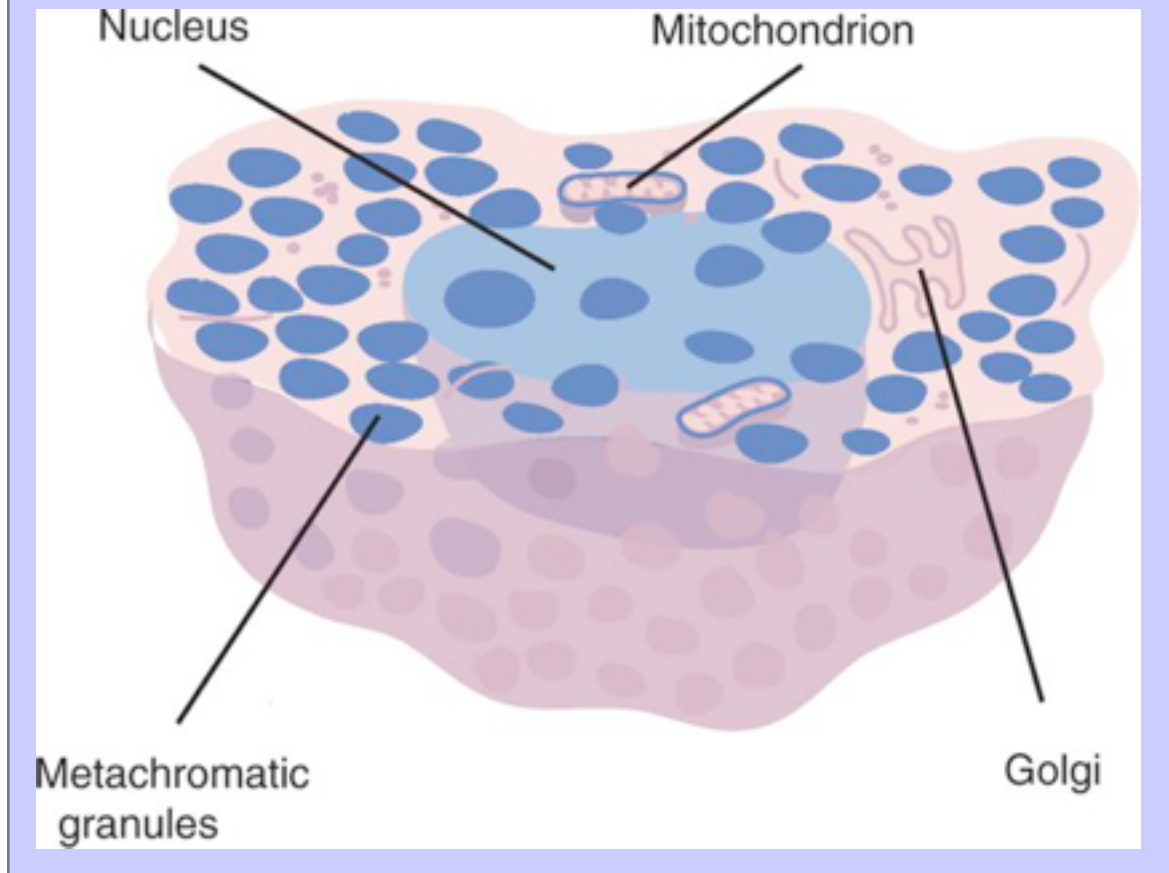


FIGURE 2-11 A diagram of the structural features of a connective tissue mast cell. The term metachromatic simply means that the granules stain intensely.



blood vessels, where they can regulate blood flow and influence cellular migration. They are easily recognizable because their cytoplasm is densely packed with large granules (secretory lysosomes) that stain very strongly with dyes such as toluidine blue. These granules often mask the large, bean-shaped nucleus ([Figure 2-11](#)). (Mast cells are so called because, being full of granules, they were considered to be “well-fed” cells (German *Mastzellen*). Mast cells from connective tissue and skin and from the intestinal walls differ both chemically and structurally ([Table 2-3](#)). For example, connective tissue and skin mast cells are rich in the molecules histamine and heparin, whereas mucosal mast cells contain chondroitin sulfate and have little histamine in their granules.

Table 2-3 Comparison of Two Major Types of Mast Cell

	Mucosal Mast Cells	Connective Tissue Mast Cells
Structure	Few, variable-sized granules	Many uniform granules
Size	9-10 µm diameter	19-20 m diameter
Proteoglycan	Chondroitin sulfate	Heparin
Histamine	1.3 pg/cell	15 pg/cell
Life span	<40 days	>6 mo
Location	Intestinal wall, lung	Peritoneal cavity, skin

2.4.3.2

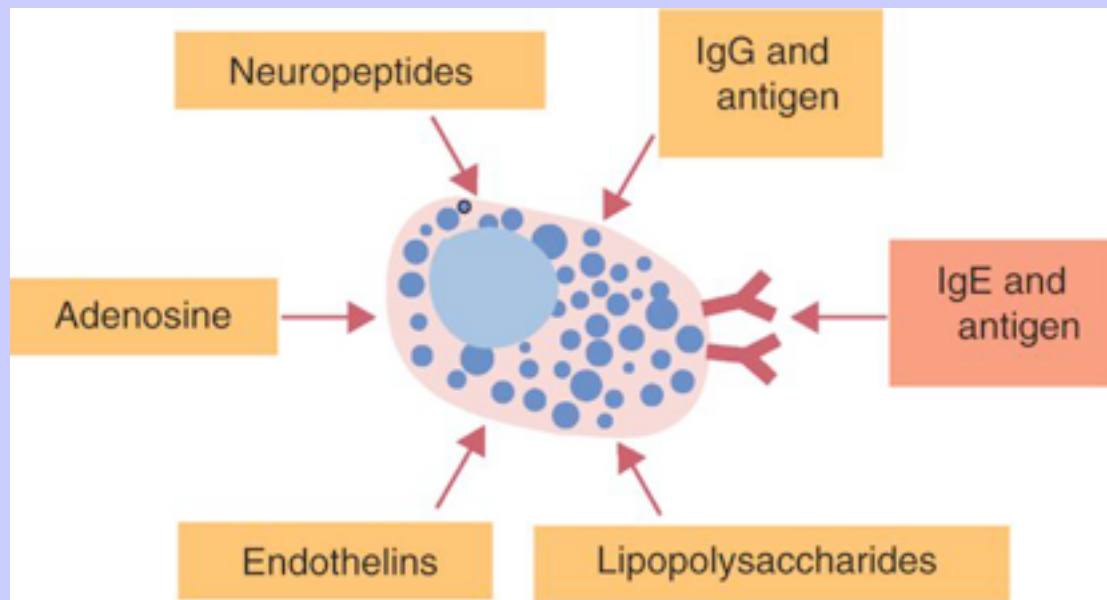
Life History

Mast cells originate from stem cells in the bone marrow. The mast cell precursors emigrate to tissues, where they mature and survive for several weeks or months. Although connective tissue mast cells remain at relatively constant levels, intestinal mast cells can proliferate. It has been suggested that the mucosal mast cells respond specifically to invasion by parasitic worms.

Mast cells have a key role in innate immunity because when they encounter invading microorganisms they release molecules that cause the changes in blood flow seen in acute inflammation. These inflammatory molecules are normally confined to the mast cell granules, but they are released when the cells degranulate. Many different mechanisms stimulate mast cell degranulation. The best recognized of these involves an antibody molecule called immunoglobulin E (IgE) (see [Chapter 25](#)). IgE and antigen together can trigger mast cell degranulation and so cause the severe inflammation that occurs in allergic diseases. However, allergies are a special case. Numerous other signals can activate mast cells including cytokines, chemokines, chemical agents, physical stimuli, various peptides, insect and animal venoms, bacteria and bacterial products, and viruses. In normal inflammation, mast cells release inflammatory mediators relatively slowly in a process called piecemeal degranulation. They may also secrete some vasoactive factors without degranulation ([Figure 2-12](#)). For example, bacteria and bacterial products can trigger mast cells to produce TNF- α , IL-1 β , and IL-6 without degranulation. Many alarmins, including the defensins, neuropeptides, adenosine, and endothelins (small peptides from endothelial cells), also trigger mast cell degranulation.

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FIGURE 2-12 Some of the stimuli that make mast cells degranulate. Antigen bound through immunoglobulin E (IgE) causes rapid, complete degranulation. The other stimuli shown cause a more gradual, piecemeal degranulation. Thus in normal inflammatory responses the degree of mast cell degranulation is tailored to local defensive needs.



Mast cells possess a wide array of pattern-recognition receptors that permit them to recognize the presence of pathogens. Thus they express TLRs 1, 2, 3, 4, 6, 7, and 9. They also possess a mannose receptor (CD48). As a result, mast cells can sense the presence of microbes and respond accordingly. Mast cells also possess receptors for molecules released by activation of the immune system such as some complement components.

Stimulation of their TLRs causes mast cells to release different mixtures of mediators. Thus bacterial peptidoglycans acting through TLR2 stimulate histamine release, whereas LPS acting through TLR4 do not. Mast cells can thus distinguish between different pathogens and generate highly selective combinations of cytokines, chemokines, and other inflammatory mediators, depending upon the stimulus they receive.

2.5 PRODUCTS OF SENTINEL CELLS

Macrophages, DCs, and mast cells are activated when PAMPs or alarmins bind to their receptors. As a result, they respond by synthesizing and secreting a mixture of cytokines and other molecules that trigger inflammation while starting to activate acquired immunity.

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2.5.1 Cytokines

When exposed to infectious agents or their PAMPs, sentinel cells synthesize and secrete many different proteins including the major cytokines IL-1 and TNF- α , as well as IL-6, IL-12, and IL-18. They synthesize nitric oxide synthase 2 (NOS2), which generates oxidants such as nitric oxide. They synthesize the enzyme cyclooxygenase-2 (COX-2) that generates the inflammatory lipids prostaglandins and leukotrienes. When released in sufficient quantities, these molecules cause a fever and sickness behavior and promote an acute-phase response (see [Chapter 4](#)). If the sentinel cells detect the presence of damaged or foreign DNA, such as that from viruses, they trigger dendritic cells to secrete the antiviral cytokines known as interferons (see [Chapter 23](#)).

2.5.1.1 Tumor Necrosis Factor- α

TNF- α is a 25 kDa trimeric protein produced by macrophages, mast cells, T cells, endothelial cells, B cells, and fibroblasts. It can occur in soluble or membrane-bound forms. The membrane-bound form is cleaved from the cell surface by a protease called TNF- α convertase. TNF- α plays a key role in triggering inflammation. Upon detecting invading pathogens, macrophages and mast cells secrete either membrane-associated TNF- α or soluble TNF- α . The TNF- α triggers local release of chemokines and cytokines and promotes the adherence, migration, attraction, and activation of leukocytes at the site of invasion. Later, TNF- α facilitates the transition from innate to acquired immunity by enhancing antigen presentation and T cell co-stimulation. Its production is stimulated not only through TLRs but also by molecules secreted by nerves such as the neurotransmitter substance P. TNF- α is produced very early in inflammation, and this is followed by waves of IL-1 and then by IL-6.

TNF- α is an essential mediator of inflammation because in combination with IL-1 it triggers changes in the cells that line small blood vessels (vascular endothelial cells). A local increase in TNF- α causes the “cardinal signs” of inflammation, including heat, swelling, pain, and redness. Systemic increases in TNF- α depress cardiac output, induce microvascular thrombosis, and cause capillary leakage. TNF- α acts on neutrophils (key defensive cells in inflammation; see [Chapter 3](#)) to enhance their ability to kill microbes. It attracts neutrophils to sites of tissue damage and increases their adherence to vascular endothelium ([Figure 2-13](#)). It stimulates macrophage phagocytosis and oxidant production. It amplifies and prolongs inflammation by promoting macrophage synthesis of IL-1, NOS2, and COX-2. TNF- α also activates mast cells.

TNF- α activates macrophages to increase its own synthesis together with that of IL-1. As its name implies, TNF- α can kill some tumor cells and virus-infected cells by activating caspases and inducing apoptosis. In high doses, TNF- α can cause septic shock.

2.5.1.2 Interleukin-1

When stimulated by CD14 and TLR4, macrophages synthesize two glycoproteins called IL-1 α and IL-1 β . IL-1 β is produced as a large pro-protein that is cleaved

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FIGURE 2-13 Some of the properties of tumor necrosis factor- α .

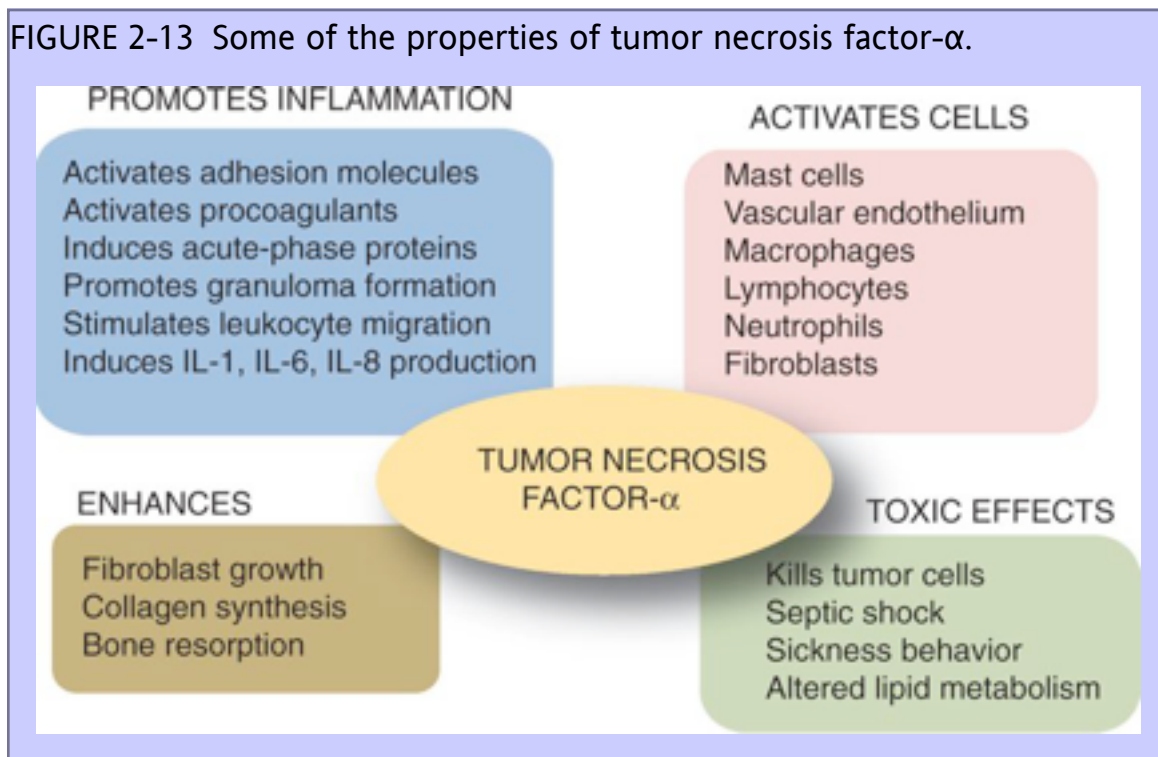
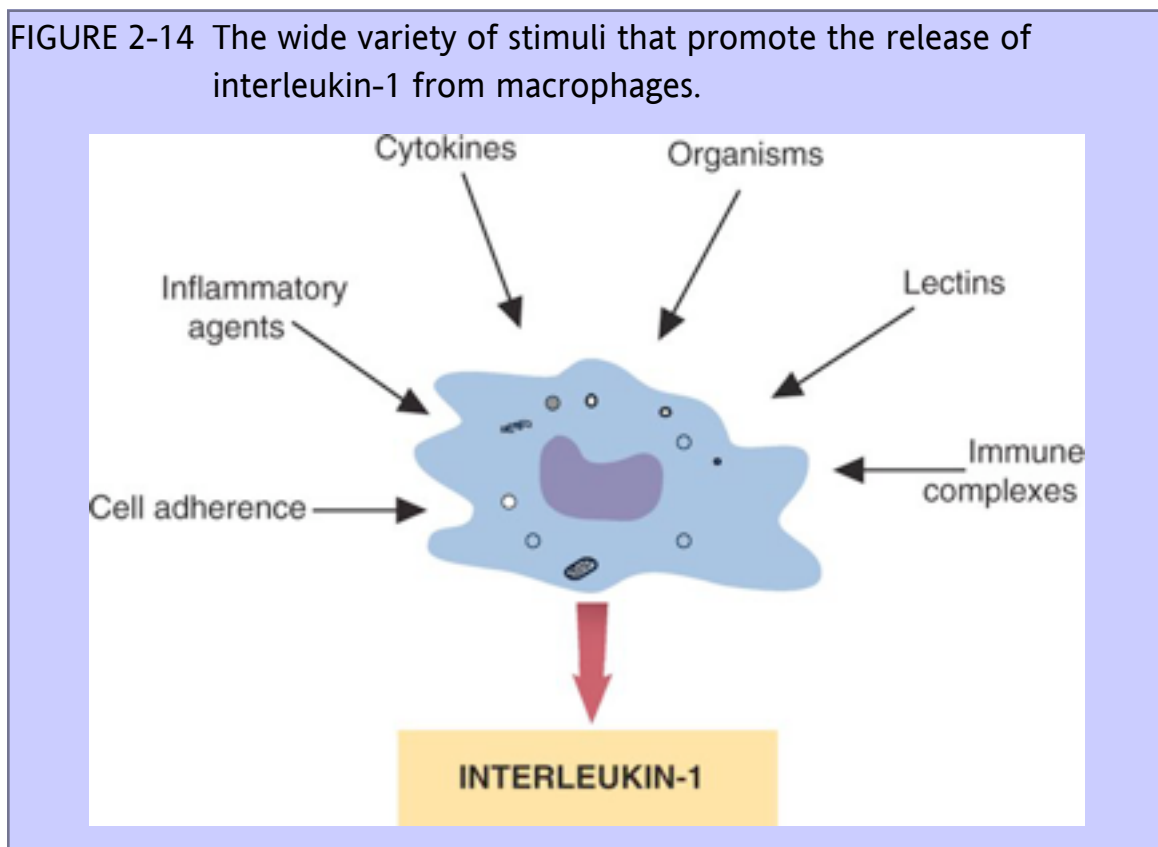


FIGURE 2-14 The wide variety of stimuli that promote the release of interleukin-1 from macrophages.



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by caspase-1 to form the active molecule. Tenfold to fiftyfold more IL-1 β is produced than IL-1 α , and while IL-1 β is secreted, IL-1 α remains attached to the cell. Therefore IL-1 α acts only on target cells that come into direct contact with the macrophage ([Figure 2-14](#)). Transcription of IL-1 β mRNA occurs within 15 minutes of ligand binding. It reaches a peak 3 to 4 hours later and levels off for several hours before declining. Like TNF- α , IL-1 β acts on vascular endothelial cells to make them adhesive for neutrophils. IL-1 acts on other macrophages to stimulate their synthesis of NOS2 and COX-2 and so promote more inflammation.

During severe infections, some IL-1 β circulates in the bloodstream, where (in association with TNF- α) it is responsible for sickness behavior. Thus it acts on the brain to cause fever, lethargy, malaise, and lack of appetite ([Figure 2-15](#)). It acts on muscle cells to mobilize amino acids causing pain and fatigue. It acts on liver cells to induce the production of new proteins, called acute-phase proteins, that assist in the defense of the body (see [Chapter 4](#)).

The most important IL-1 receptors are CD121a and CD121b. CD121a is a signaling receptor, whereas CD121b is not. CD121b thus inhibits IL-1 functions. Soluble CD121b can bind IL-1 and so acts as an IL-1 antagonist. IL-1 receptor antagonist (IL-1RA) is an inactive molecule that binds and blocks CD121a. IL-1RA is therefore an important regulator of IL-1 activity and inflammation. It reduces mortality in septic shock and graft-versus-host disease (see [Chapter 4](#)) and has antiinflammatory effects.

2.5.1.3

Interleukin-6

IL-6 is also produced by macrophages and mast cells. Its production is stimulated by the bacterial endotoxins, IL-1 and TNF- α . IL-6 affects both inflammation and acquired immunity. It is a major mediator of the acute-phase reaction and of septic shock (see [Chapter 4](#)). It has been suggested that IL-6 regulates the transition from a neutrophil-dominated process early in inflammation to a macrophage-dominated process later on.

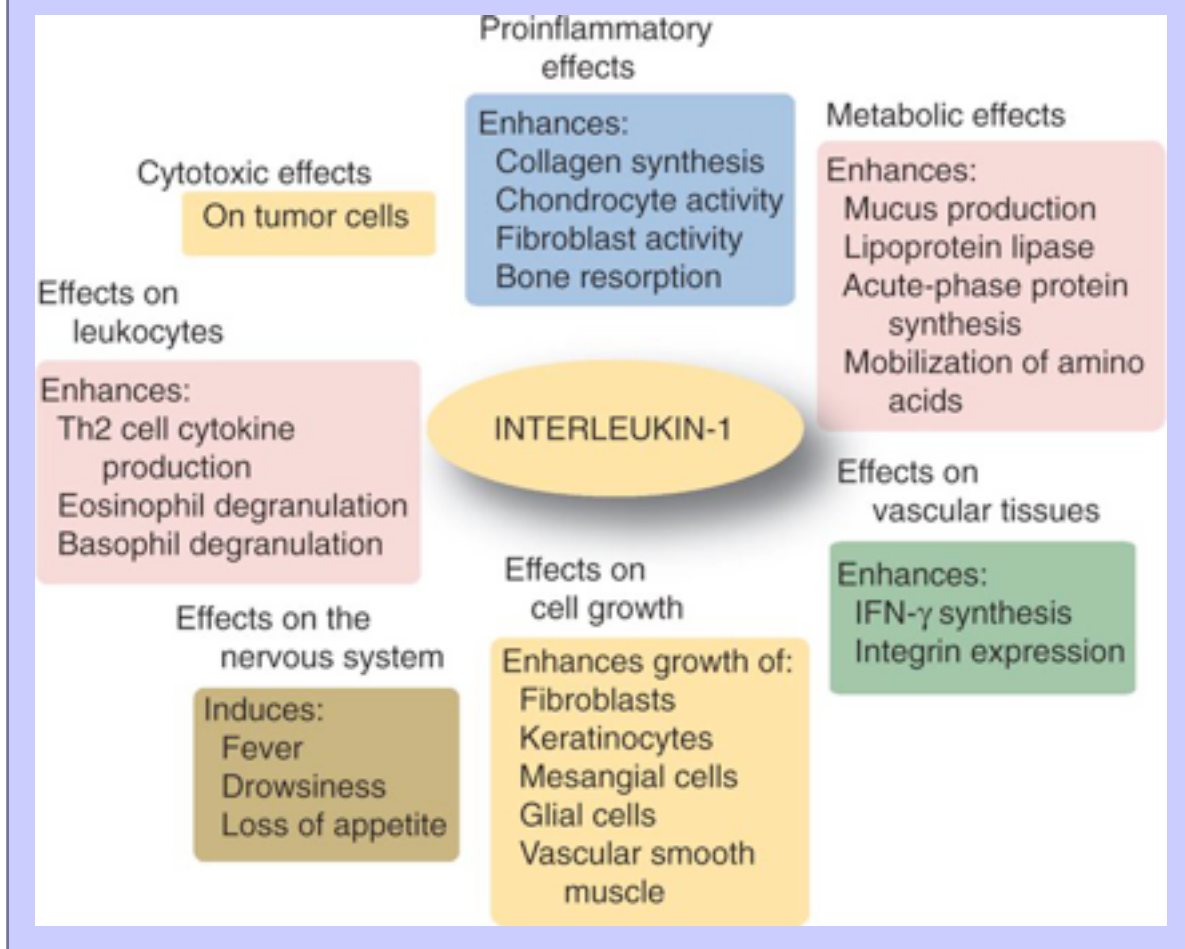
2.5.2

Chemokines

Chemokines are a family of small (8 to 10 kDa) proteins that control cellular migration. Because they regulate the movement of specific cell populations, they can dictate the course of many inflammatory and immune responses ([Table 2-4](#)). Chemokines are produced by diverse cell types including macrophages and mast cells. At least 50 different chemokines have been identified. They are classified into four families according to the spacing of their cysteine residues ([Figure 2-16](#)). For example, the CC, or α , chemokines have two contiguous cysteine residues, whereas the CXC, or β , chemokines have two cysteine residues separated by another amino acid. (Chemokine nomenclature is based on this classification, each molecule or receptor receiving a numerical designation. Ligands have the

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FIGURE 2-15 Some of the effects of interleukin-1 on the cells of the body.



suffix "L" [e.g., CXCL8], whereas receptors have the suffix "R" [e.g., CXCR1].)

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Table 2-4 The Nomenclature of Some Selected Chemokines and Their Receptors

New Name	Old Name	Receptor
α Family		
CCL2	MCP-1	CCR2
CCL3	MIP-1 α	CCR1, CCR5
CCL4	MIP-1 β	CCR5
CCL5	RANTES	CCR1, CCR3, CCR5
CCL7	MCP-3	CCR3
CCL8	MCP-2	CCR3
CCL11	Eotaxin	CCR3
CCL13	MCP-4	CCR3
CCL20	MIP-3 α	CCR6
CCL22	MDC	CCR4
CCL26	Eotaxin 3	CCR3
CCL28	MEC	CCR3
β Family		
CXCL1	GRO1	CXCR2
CXCL7	MDGF	CXCR2
CXCL8	IL-8	CXCR1, CXCR2
CXCL12	SDF	CXCR4
CXCL13	BCA-1	CXCR5
γ Family		
XCL1	Lymphotactin	XCR1
δ Family		
CX3CL1	Fractalkine	CX3CR1

CXCL8 (or IL-8) is a typical example of a CXC chemokine produced by stimulation of macrophages or mast cells. CXCL8 will attract and activate neutrophils, releasing their granule contents and stimulating the respiratory burst and leukotriene release (see [Chapter 3](#)). Another important CXC chemokine is CXCL2 (macrophage inflammatory protein-2, MIP-2), which is secreted by macrophages and also attracts neutrophils.

CC chemokines act predominantly on macrophages and DCs. Thus CCL3 and CCL4 (MIP-1a and MIP-1b) are produced by macrophages and mast cells. CCL4 attracts CD4⁺ T cells, whereas CCL3 attracts B cells, eosinophils, and cytotoxic T cells. CCL2 (monocyte chemoattractant protein-1, MCP-1) is produced by macrophages, T cells, fibroblasts, keratinocytes, and endothelial cells. It attracts and activates monocytes, stimulating their

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respiratory burst and lysosomal enzyme release. CCL5 (RANTES [regulated on activation normal T cell expressed and secreted]) is produced by T cells and macrophages. It is chemotactic for monocytes, eosinophils, and some T cells. It activates eosinophils and stimulates histamine release from basophils. Regakine-1 is a CC chemokine found in bovine serum that acts together with CXCL8 and C5a to attract neutrophils and enhance inflammation.

Two chemokines fall outside the CC and CXC families. A C (only one cysteine residue) or γ chemokine, called XCL1 (or lymphotactin), is chemotactic for lymphocytes. Its receptor is XCR1. The CXXXX (two cysteines separated by three amino acids) or δ chemokine called CX3CL1 (or fractalkine) triggers adhesion by T cells and monocytes. Its receptor is CX3CR1.

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Most chemokines are produced in inflamed or damaged tissues and attract other cells to sites of inflammation or microbial invasion. It is probable that several different chemokines serve to attract different cell types to inflammatory sites. Indeed it is likely that the chemokine mixture produced in damaged tissues regulates the precise composition of the inflammatory cell populations. In this way the body can adjust the inflammatory response to provide the most effective way of destroying different microbial invaders. Many chemokines, such as CXCL4, CCL20, and CCL5, are structurally similar to the antimicrobial proteins called defensins and, like them, have significant antibacterial activity. Chemokines have a major role in infections and inflammation in domestic animal species. They regulate immune cell trafficking. They have been detected in many inflammatory diseases, including pneumonia (bovine pasteurellosis), bacterial mastitis, arthritis, and endotoxemia. Impaired neutrophil migration is associated with specific CXCR2 genotypes and may lead to increased susceptibility to bovine mastitis.

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2.6 INCREASED VASCULAR PERMEABILITY

Acute inflammation can develop within minutes after a tissue is damaged. The damaged tissue triggers three types of signal. First, broken cells release alarmins that trigger the release of cytokines from sentinel cells. Second, microbes provide PAMPs that trigger sentinel cell responses, including the production of cytokines and other inflammatory mediators. Third, pain causes nerves to release bioactive peptides.

In its classical form, acute inflammation has five cardinal signs: heat, redness, swelling, pain, and loss of function. All these signs result from changes in small blood vessels ([Figure 2-17](#)). Immediately after injury the blood flow through small capillaries at the injection site is decreased to give leukocytes an opportunity to bind to the blood vessel walls. Shortly thereafter, the small blood vessels in the damaged area dilate and blood flow to the injured tissue increases. While the blood vessels are dilated, they also leak so that fluid moves from the blood into the tissues where it causes edema and swelling.

At the same time as these changes in blood flow are occurring, cellular responses are taking place. The changes in cells lining blood vessel walls permit neutrophils and monocytes to adhere to the vascular endothelial cells. If the blood vessels are damaged, blood platelets may also bind to the injured sites and release vasoactive and clotting molecules.

Inflamed tissues swell as a result of leakage of fluid from blood vessels. This leakage occurs in two stages. First there is an immediate increase in leakage mediated by vasoactive molecules released by mast cells, by damaged tissues, and by nerves ([Table 2-5](#)). The second phase of increased leakage occurs several hours after the onset of inflammation, at a time when the leukocytes are beginning to emigrate. Endothelial and perivascular cells contract so that they pull apart and allow fluid to escape through the intercellular spaces.

2.7 VASOACTIVE MOLECULES

Mast cells respond to signals from damaged tissues by releasing a mixture of molecules that affect blood vessel walls (vasoactive molecules). These include histamine, vasoactive lipids, enzymes (tryptase and

FIGURE 2-16 The classification of chemokines is based on the location and spacing of their cysteine residues.

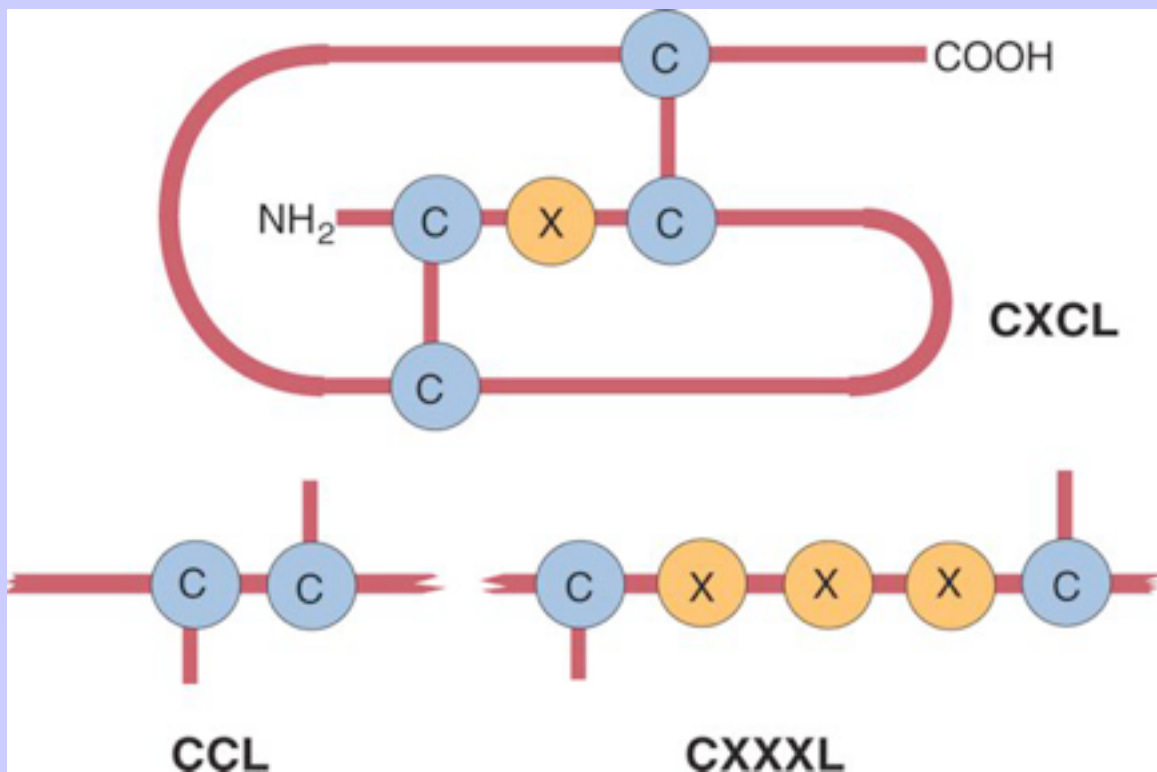
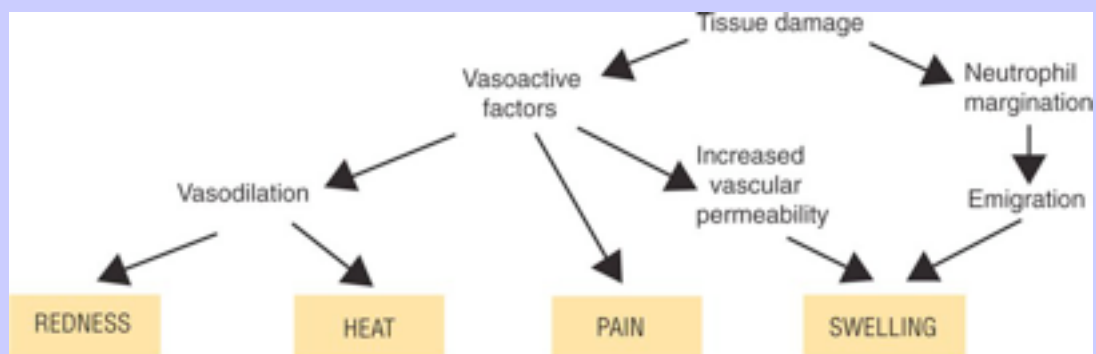


FIGURE 2-17 The cardinal signs of acute inflammation and how they are generated.



chymase), cytokines, and chemokines. Histamine, the lipids, and tryptases cause vasodilation and blood vessel leakage of fluid. The tryptases activate receptors on mast cells, sensory nerve endings, vascular endothelial cells, and neutrophils. As a result, the blood vessel walls become sticky for neutrophils. Activated neutrophils release a lipid called platelet-activating factor (PAF). The PAF makes endothelial cells even stickier and so enhances neutrophil adhesion and emigration. PAF is a phospholipid closely related to lecithin. It is synthesized by mast cells, platelets, neutrophils, and eosinophils. PAF aggregates platelets and makes them release their vasoactive molecules and synthesize thromboxanes. It acts on neutrophils in a similar fashion. Thus it promotes neutrophil aggregation, degranulation, chemotaxis, and release of oxidants.

Table 2-5 Some Vasoactive Molecules Produced during Acute Inflammation

Mediator	Major Source	Function
Histamine	Mast cells and basophils, platelets	Increased vascular permeability, pain
Serotonin	Platelets, mast cells, basophils	Increased vascular permeability
Kinins	Plasma kininogens and tissues	Vasodilation Increased vascular permeability, pain
Prostaglandins	Arachidonic acid	Vasodilation, increased vascular permeability
Thromboxanes	Arachidonic acid	Increased platelet aggregation
Leukotriene B ₄	Arachidonic acid	Neutrophil chemotaxis Increased vascular permeability
Leukotrienes C, D, E	Arachidonic acid	Smooth muscle contraction Increased vascular permeability
Platelet-activating factor	Phagocytic cells	Platelet secretion Neutrophil secretion Increased vascular permeability
Fibrinogen breakdown products	Clotted blood	Smooth muscle Neutrophil chemotaxis Increased vascular permeability
C3a and C5a	Serum complement	Mast cell degranulation Smooth muscle contraction Neutrophil chemotaxis (C5a)

The most important of the vasoactive molecules released by mast cells is histamine ([Figure 2-18](#)). The effects of histamine are mediated through multiple receptors. H1 and H2 receptors are expressed on nerve cells, smooth muscle cells, endothelial cells, neutrophils, eosinophils, monocytes, DCs, and T and B cells. Histamine binding to H1 receptors stimulates endothelial cells to produce nitric oxide, a very potent vasodilator. At the same time, histamine causes vascular leakage, leading to fluid accumulation and local edema. Histamine upregulates TLR expression on sentinel cells.

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Serotonin (5-hydroxytryptamine [5-HT]), a derivative of the amino acid tryptophan, is released from the mast cells of some rodents and the large domestic herbivores. Serotonin normally causes a vasoconstriction that results in a rise in blood pressure (except in cattle, in which it is a vasodilator). It has little effect on vascular permeability, except in rodents, where it induces acute inflammation.

Although histamine and serotonin are very important mediators of inflammation, they are only a fraction of the complex mixture of molecules released when mast cells degranulate. More than half the protein in mast cell granules consists of proteases called tryptases and chymases. These enzymes sensitize smooth muscle to histamine; they stimulate proliferation of fibroblasts, smooth muscle, and epithelial cells; generate kinins; increase expression of adherence proteins; and stimulate the release of the chemokine CXCL8. Mast cell tryptases can also activate some receptors on sensory nerves, neutrophils, mast cells, and endothelial cells.

2.7.1

Vasoactive Lipids

When tissues are damaged or stimulated, phospholipases act on cell wall phospholipids to release arachidonic acid. Under the influence of the enzyme 5-lipoxygenase, the arachidonic acid is converted to biologically active lipids called leukotrienes ([Figure 2-19](#)).

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FIGURE 2-18 Structure of some major vasoactive molecules active during acute inflammation.

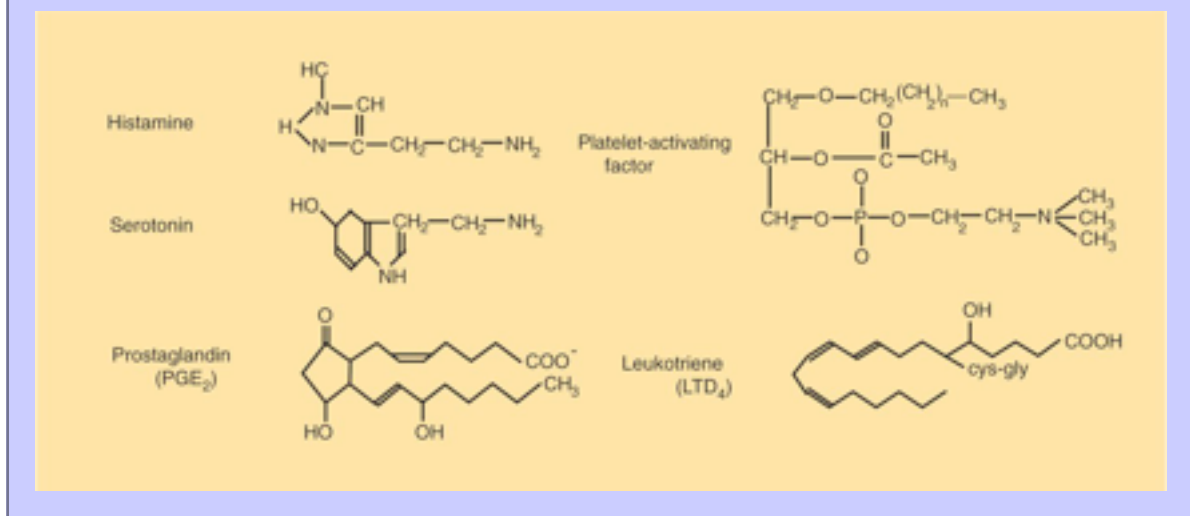
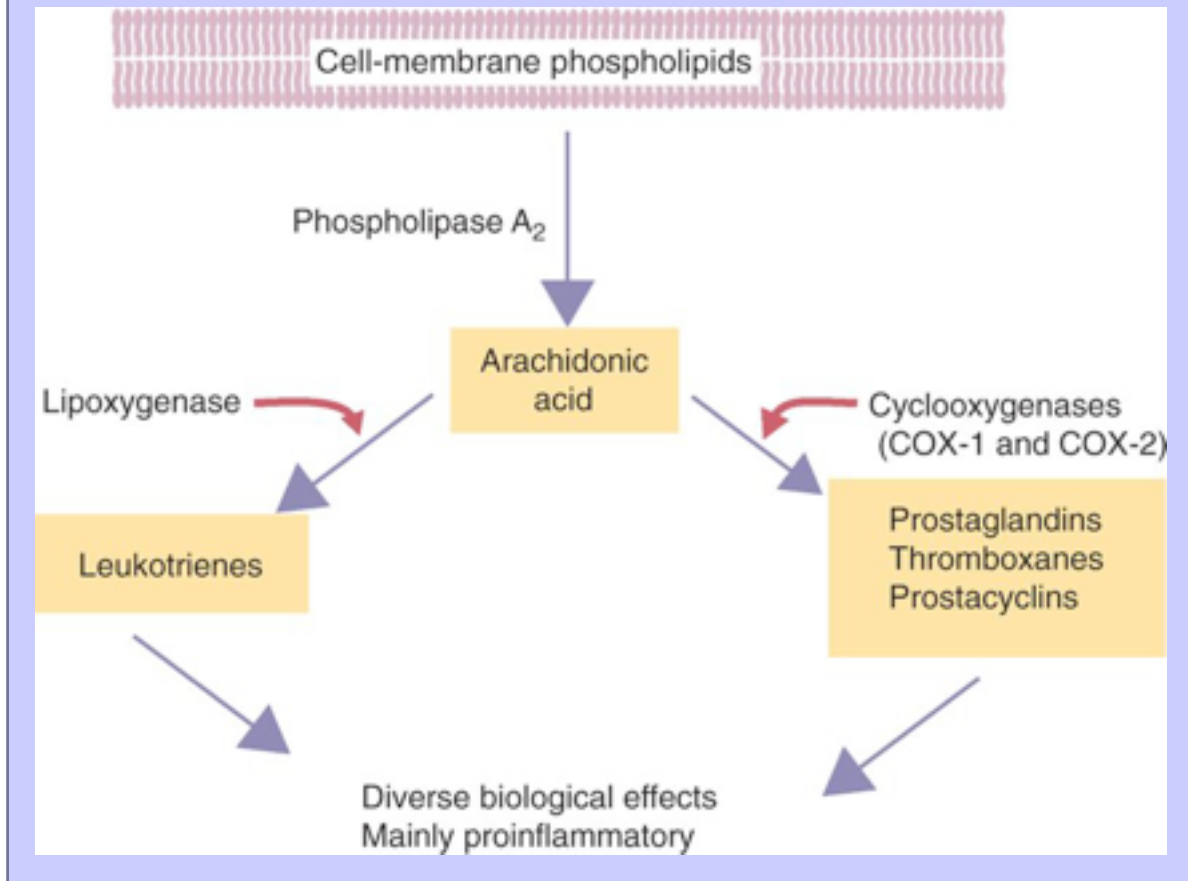


FIGURE 2-19 Production of leukotrienes and prostaglandins by the actions of lipoxygenase and cyclooxygenase (COX) on arachidonic acid. Both prostaglandins and leukotrienes may have proinflammatory or antiinflammatory activity depending on their chemical structure.



Under the influence of cyclooxygenases, arachidonic acid is converted to a second group of active lipids called prostaglandins. The collective term for all these complex lipids is eicosanoids.

Four leukotrienes play a central role in inflammation. Leukotriene B₄ (LTB₄) is an especially potent neutrophil attractant and is probably one of the most important mediators released by mast cells during bacterial infections. LTB₄ also stimulates eosinophil chemotaxis and random motility. Leukotrienes C₄, D₄, and E₄, in contrast, increase vascular permeability.

There are four groups of proinflammatory prostaglandins: PGE₂, PGF₂, the thromboxanes (TxA₂, PGA₂), and the prostacyclins (PGI₂). The enzymes that generate the prostacyclins are found in vascular endothelial cells, the thromboxanes are found in platelets, and the other prostaglandins can be generated by most nucleated cells. The biological activities of the prostaglandins vary widely, and since many different prostaglandins are released in inflamed tissues, their net effect may be very complex.

As neutrophils enter inflammatory sites, they use the enzyme 15-lipoxygenase to produce lipoxins from arachidonic acid. These oxidized eicosanoids bind cellular receptors and inhibit neutrophil migration. Thus there is a gradual switch in production from proinflammatory leukotrienes to antiinflammatory lipoxins. The rise in PGE₂ in tissues also gradually inhibits 5-lipoxygenase activity and so eventually suppresses inflammation.

2.7.2

Vasoactive Peptides

Mast cell proteases act on the complement components C3 and C5 to generate two small, biologically active peptides—C3a and C5a (see [Chapter 5](#)). Both promote histamine release from mast cells. In addition, C5a is a very potent attractant for neutrophils and monocytes. Mast cell granules also contain proteases called kallikreins. These act on proteins called kininogens to generate small peptides called kinins. Both the kinins and the anaphylatoxins cause blood vessel dilation and leakage. The most important of the kinins is bradykinin. Kinins not only increase vascular permeability, they also stimulate neutrophils and trigger pain receptors and they may have defensin-like antimicrobial activity.

2.8

THE COAGULATION SYSTEM

When fluid leaks from the bloodstream into the tissues, blood coagulation is activated. Platelet aggregation accelerates this process. Activation of the coagulation system generates large quantities of thrombin, the main clotting enzyme. Thrombin acts on fibrinogen in tissue fluid and plasma to produce insoluble fibrin. Fibrin is therefore deposited in inflamed tissues, where it forms an effective barrier to the spread of infection. Activation of the coagulation cascade also initiates the fibrinolytic system. This leads to activation of plasminogen activator, which in turn generates plasmin, a potent fibrinolytic enzyme. In destroying fibrin, plasmin releases peptide fragments that are chemotactic for neutrophils.

2.9

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³ CHAPTER 3 Neutrophils and Their Products

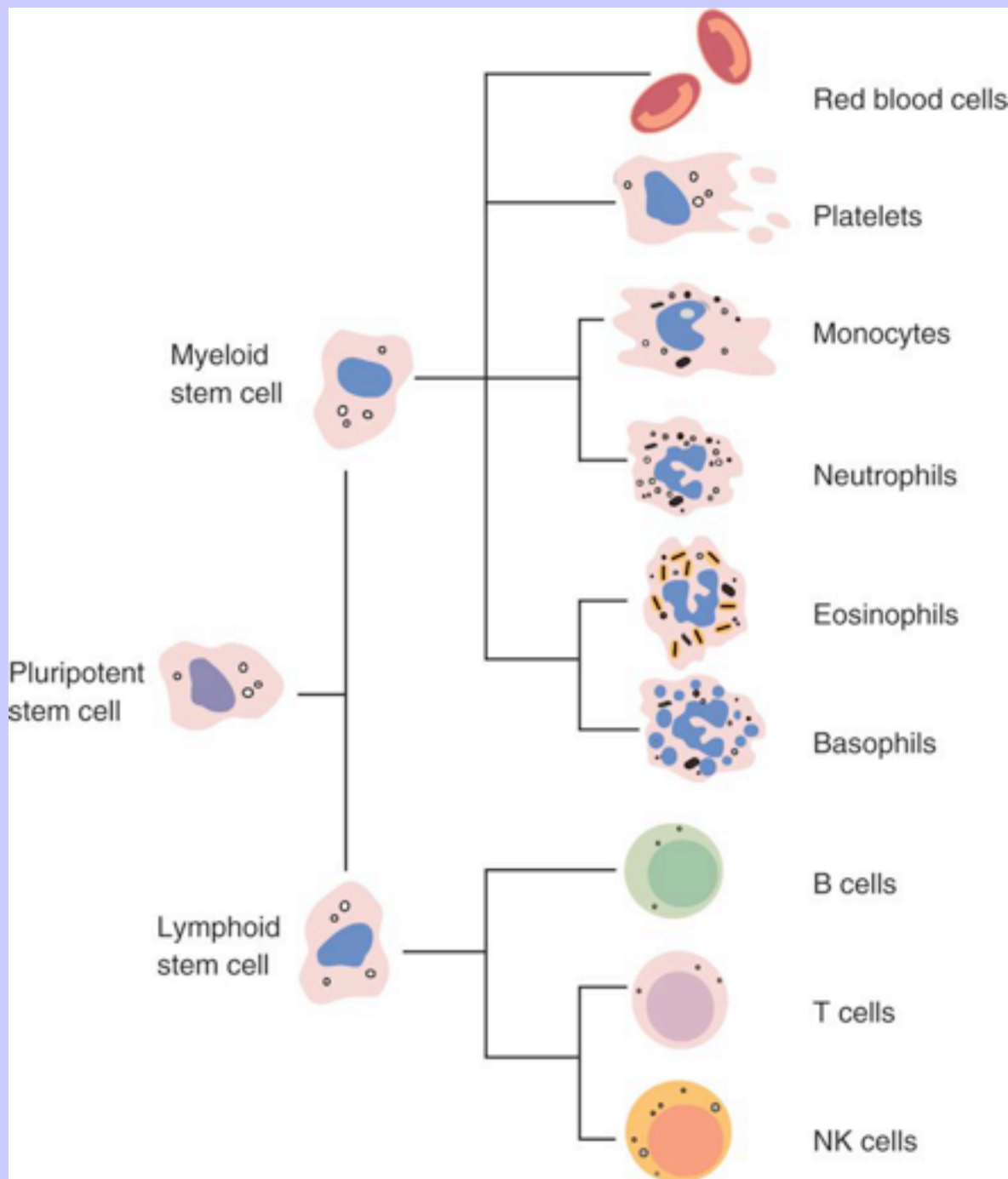
3.1 KEY POINTS

- The major cell type attracted to sites of inflammation is the neutrophil.
- Inflammatory cytokines activate vascular endothelial cells so that neutrophils in the bloodstream will adhere to them before migrating towards sites of microbial invasion and tissue damage.
- Neutrophils will bind and phagocytose invading microorganisms.
- Microorganisms need to be opsonized before they can be efficiently ingested and killed. The most effective opsonins are antibodies and complement.
- Ingested microorganisms are killed by potent oxidants through a process called the respiratory burst, by antibacterial proteins called defensins and by lytic enzymes.
- Neutrophils are short-lived cells that cannot undertake prolonged or multiple phagocytosis.

Although physical barriers such as the skin exclude many organisms, such barriers are not impenetrable, and microbial invaders often gain access to body tissues. These invaders must be promptly attacked and destroyed. Some are killed by antimicrobial peptides or complement, but many are eaten and killed by cells. This eating of microbes by cells is called phagocytosis (Greek for “eating by cells”). Phagocytosis is central to the whole inflammatory process.

The defensive cells of the body circulate in the bloodstream, where they are called leukocytes (white cells). The blood cells of mammals derive from myeloid stem cells located in the bone marrow (*myelos* is Greek for “bone marrow”) ([Figure 3-1](#)). All types of leukocyte originate from myeloid stem cells, including neutrophils, monocytes, lymphocytes, and dendritic cells, and all help defend the body. Two types of leukocytes are specialized for killing and eating invading microorganisms. These cells, called neutrophils and macrophages, originate from a common stem cell but look very different and have different, but complementary, roles. Thus neutrophils respond and eat invading organisms very rapidly but are incapable of sustained phagocytic effort. Macrophages, in contrast, move more slowly but are highly effective phagocytes and are capable of repeated phagocytosis. In this chapter we will review the properties of neu

FIGURE 3-1 The origin of cells from the bone marrow. Note that lymphoid cells originate from different stem cells than the cells of the myeloid system. Note too that cells such as eosinophils and basophils are probably closely related despite significant morphological differences.



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trophils and their role in inflammation and innate immunity.

3.2 LEUKOCYTE CLASSIFICATION

Examination of a stained blood smear reveals that there are several different types of leukocyte. Some leukocytes have a cytoplasm filled with granules; these are called granulocytes ([Figure 3-2](#)). Granulocytes have characteristic lobulated, irregular nuclei and are thus described as “polymorphonuclear” (as opposed to the single, rounded nuclei of “mononuclear” cells such as macrophages). Granulocytes are classified into three populations based on the staining properties of their granules. Cells whose granules take up basic dyes such as hematoxylin are called basophils; those whose granules take up acidic dyes such as eosin are called eosinophils; and those that take up neither basic nor acidic dyes are called neutrophils. All play important roles in the defense of the body.

3.3 NEUTROPHILS

The major cell blood leukocyte is the polymorphonuclear neutrophil granulocyte, otherwise called the neutrophil ([Figure 3-3](#)). Neutrophils are formed from stem cells in the bone marrow at a rate of about 8 million per minute in humans, migrate to the bloodstream, and about 12 hours later move into the tissues. They die after a few days and must therefore be constantly replaced. Neutrophils constitute about 60% to 75% of the blood leukocytes in most carnivores but only about 50% in the horse and 20% to 30% in cattle, sheep, and laboratory rodents. There are two pools of neutrophils in blood: a circulating pool and a pool of cells sequestered in capillaries. During bacterial infections the numbers of circulating neutrophils may increase tenfold as they are released from the bone marrow and the sequestered pool.

Toll-like receptors are expressed on myeloid stem cells. Binding to these receptors, microbial pathogen-associated molecular patterns such as lipopolysaccharides (LPS) trigger them to produce more

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FIGURE 3-2 Differentiation and nomenclature of the cells found in blood. Thus leukocytes are first differentiated on the basis of their nuclear shape. Polymorphonuclear cells are then differentiated on the basis of their granule staining. Lymphocytes and macrophages are differentiated on the basis of nuclear shape and extent of cytoplasm. Note that it is not possible to differentiate the different subpopulations of lymphocytes on the basis of their morphology.

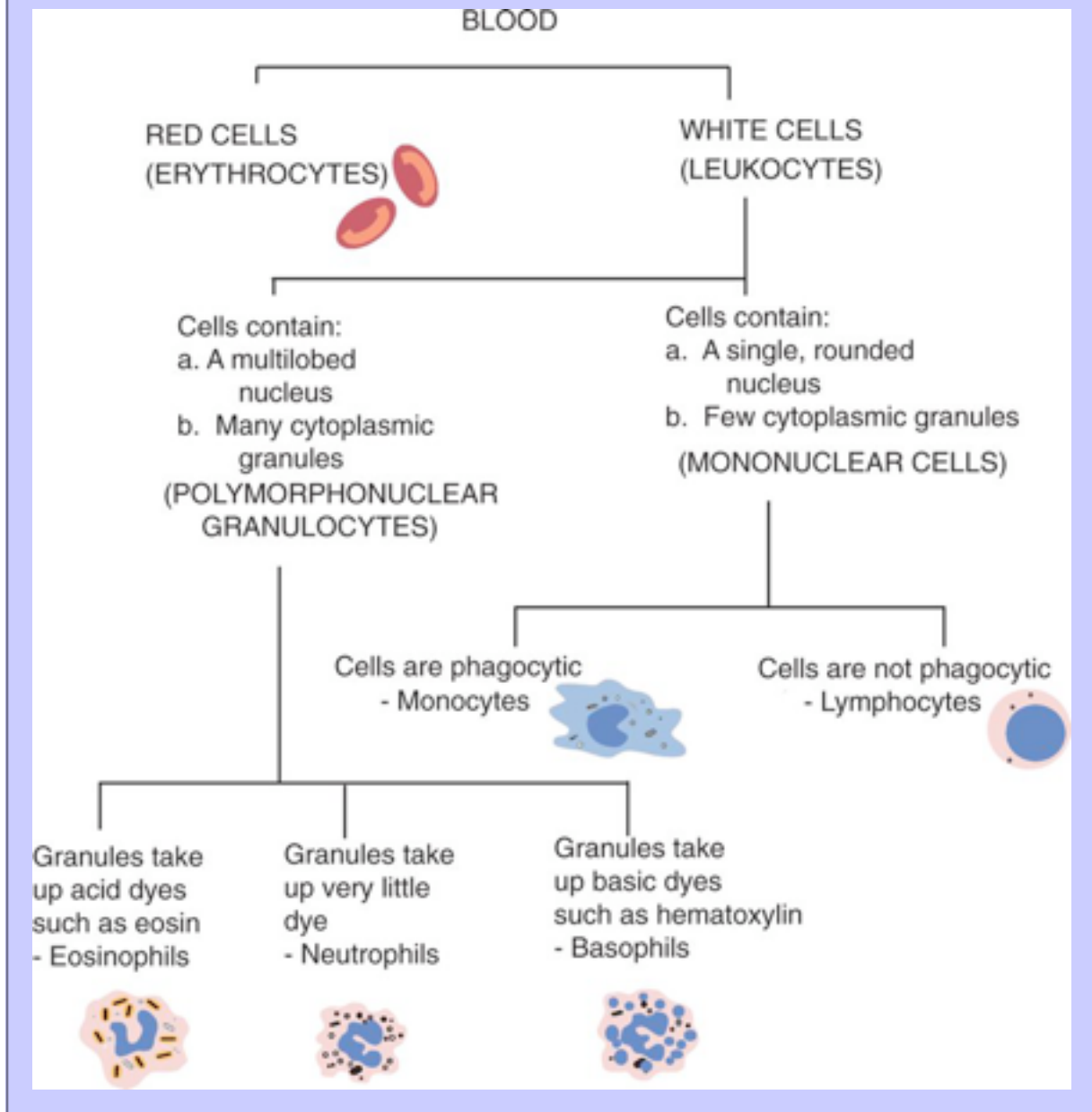
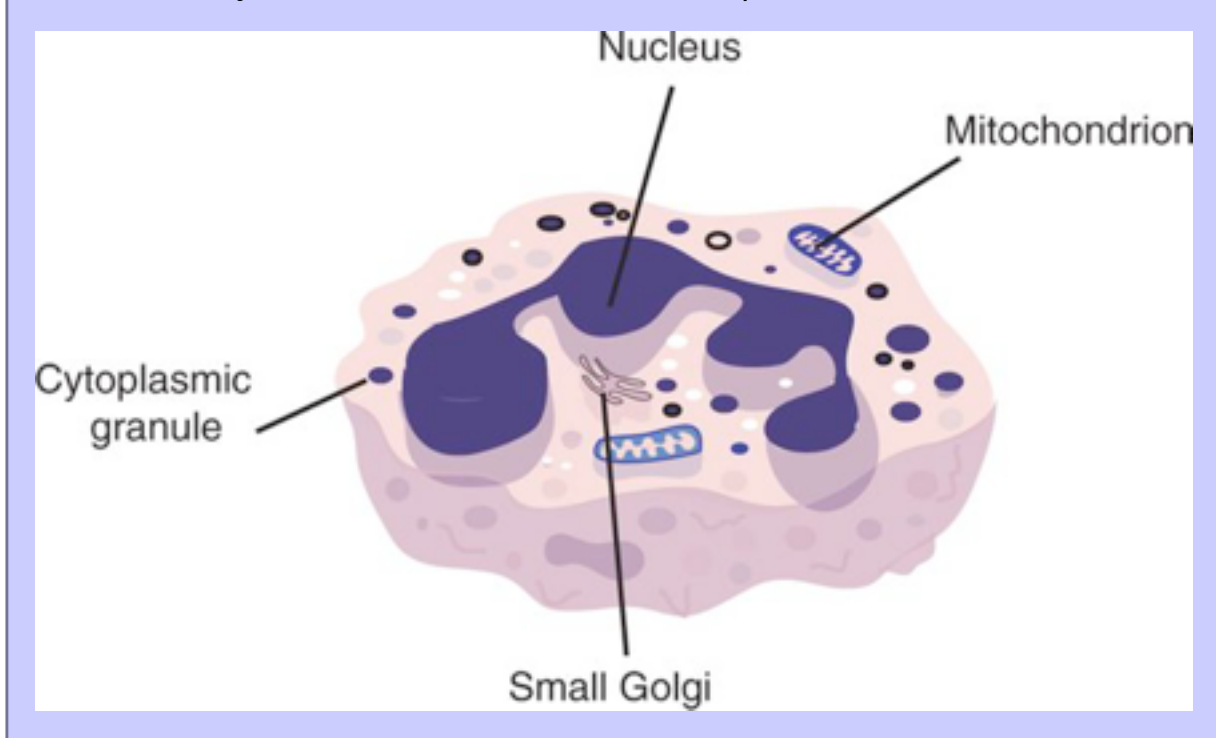


FIGURE 3-3 Major structural features of a neutrophil.



neutrophils. Toll-like receptors thus provide a pathway whereby the cells of the innate immune system may be rapidly replenished in response to infections.

3.3.1 Structure

Neutrophils suspended in blood are round cells about 10 to 20 μm in diameter. They have a finely granular cytoplasm at the center of which is an irregular sausage-like or segmented nucleus ([Figure 3-4](#)). Because the chromatin in the nucleus is compacted, neutrophils cannot divide. Electron microscopy shows many different types of enzyme-rich granules in their cytoplasm ([Figure 3-5](#)). Some of these granules contain enzymes such as myeloperoxidase, lysozyme, elastase, β -glucuronidase, and cathepsin B. Other granules lack myeloperoxidase but contain lysozyme and collagenase and the iron-binding protein lactoferrin. Mature neutrophils have a small Golgi apparatus, some mitochondria, and a few ribosomes or rough endoplasmic reticulum.

3.4 CHANGES IN VASCULAR ADHERENCE

Neutrophils are normally confined to the bloodstream and circulate with the other blood cells. If they are to defend tissues against a microbial invasion, they must leave the bloodstream. In normal tissues, neutrophils are carried along by the flow, like other blood cells. In

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FIGURE 3-4 Neutrophils in peripheral blood smears. **A**, Horse. **B**, Cat. **C**, Dog. These cells are about 10 μm in diameter. Giemsa stain. (Courtesy Dr. M.C. Johnson.)

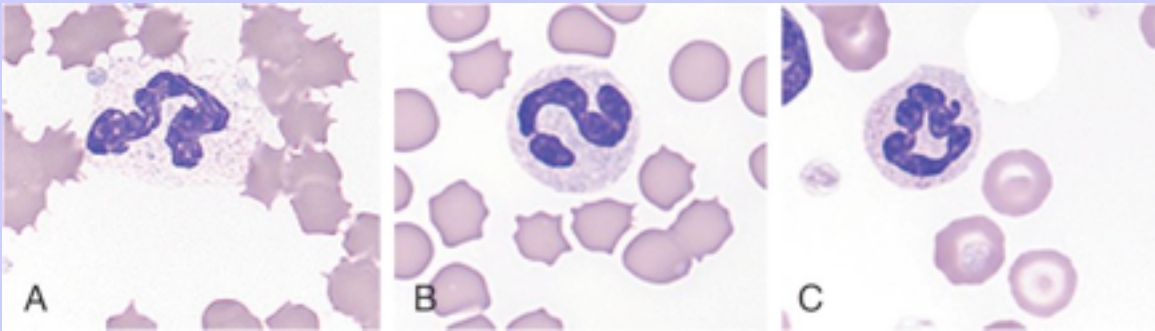


FIGURE 3-5 Transmission electron micrograph of a rabbit neutrophil. Note the two lobes of the nucleus and the granule-filled cytoplasm. (Courtesy Dr. S. Linthicum.)

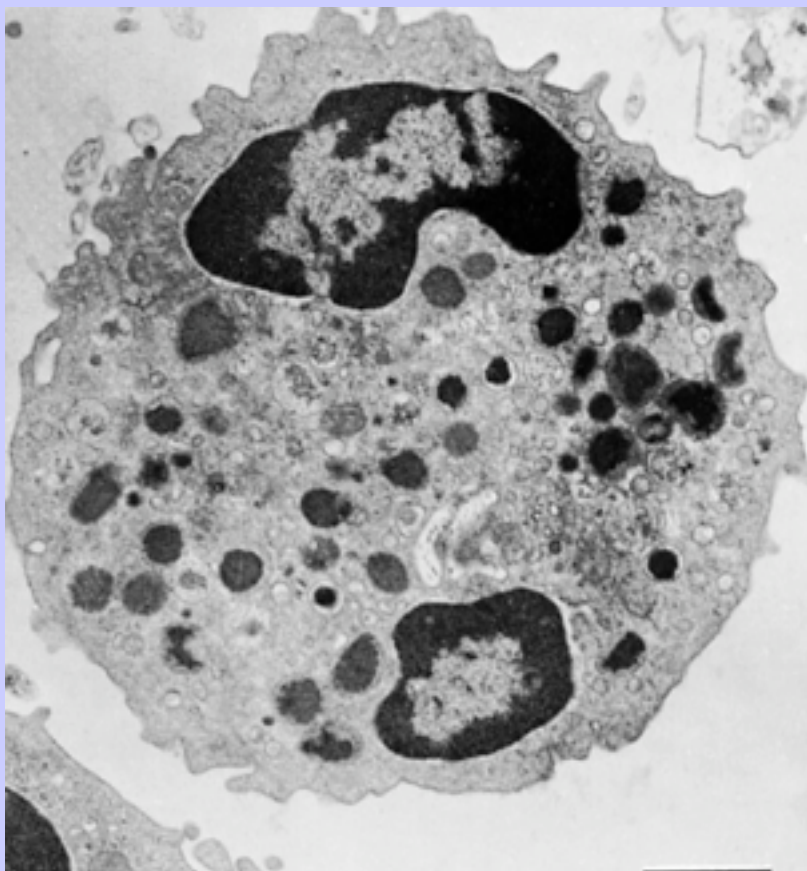
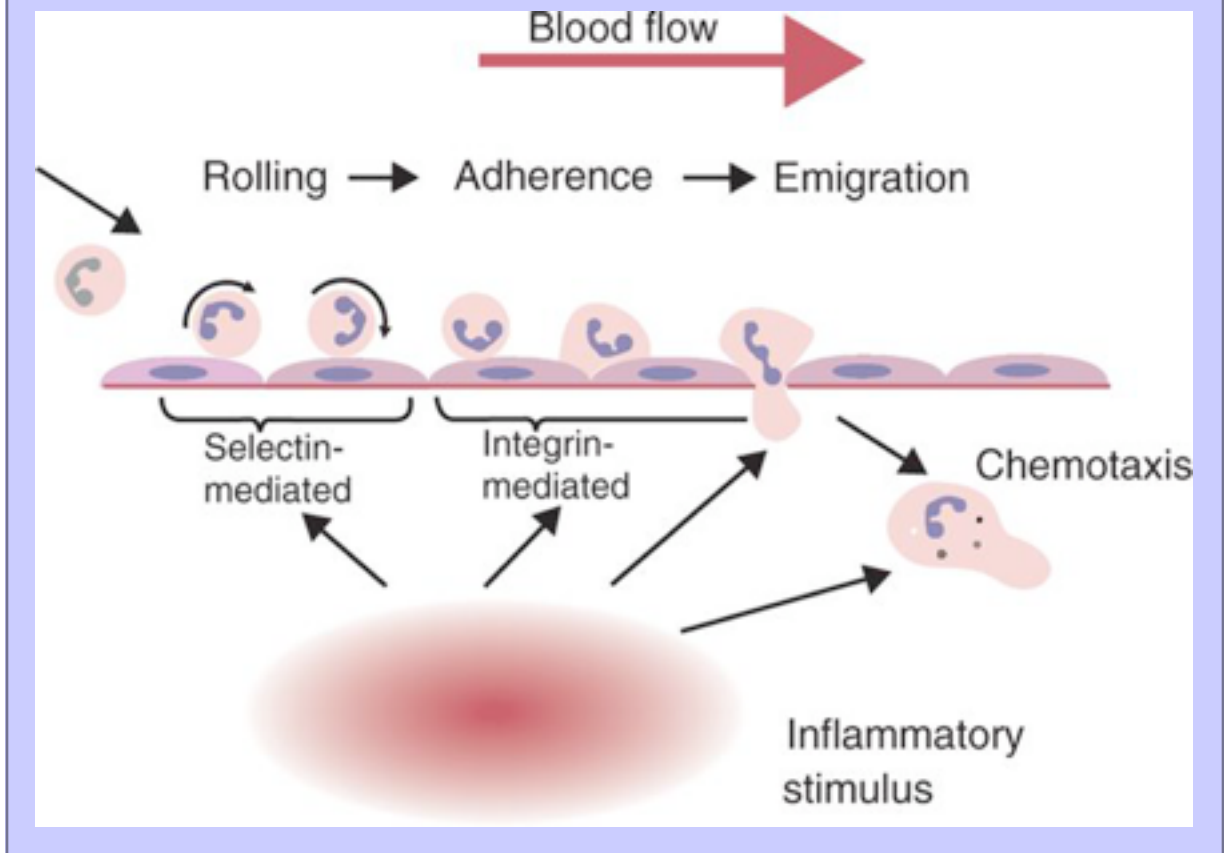


FIGURE 3-6 Stages of neutrophil adhesion and emigration from blood vessels. Selectins on endothelial cells tether neutrophils and stimulate them to roll. When they come to a halt, integrins bind them firmly to vascular endothelial cells and signal them to emigrate into tissues.



inflamed tissues these fast-moving cells must slow down, stop, bind to blood vessel walls, and then leave blood vessels by emigration through the vessel walls. This emigration is triggered by changes both in the endothelial cells that line blood vessel walls and in the neutrophils themselves.

3.4.1

Changes in Endothelial Cells

Bacterial products such as LPS, or alarmins from damaged tissues such as thrombin or histamine, cause capillary endothelial cells to express a glycoprotein called P-selectin (CD62P). P-selectin is normally stored in granules, but it moves to the endothelial cell surface within minutes after cell stimulation. Once expressed on the endothelial cell surface, the P-selectin binds to a protein called L-selectin (CD62L) on the surface of passing neutrophils. This binding is transient because the neutrophils readily shed their L-selectin. Nevertheless, the neutrophils gradually slow down and roll along the endothelial cell surface as they lose speed and eventually come to a complete stop ([Figure 3-6](#)).

3.4.2

Changes in Neutrophils

As the neutrophils roll along the endothelial surface, the second stage of adhesion occurs. Platelet-activating factor secreted by the endothelial cells activates the rolling neutrophils so that they express a protein called CD11a/CD18 or LFA-1 (leukocyte function-associated antigen-1). LFA-1 is an adhesive protein or integrin, and it binds strongly to a glycoprotein called intercellular adhesion molecule-1 (ICAM-1 or CD54) expressed on the endothelial cells. This strong binding makes the neutrophil come to a complete stop and attaches it firmly to the vessel wall despite the shearing force of the blood flow. Adherent neutrophils also secrete small amounts of elastase. The elastase removes CD43 (leukosialin), an antiadhesive protein, from the neutrophil surface, which allows

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FIGURE 3-7 Simplified view of the proteins and their ligands engaged in neutrophil-vascular endothelial cell binding. Selectins are carbohydrate-binding proteins that bind other glycoproteins through a carbohydrate called sialyl Lewis^x (SLe^x). This selectin-mediated binding is weak and temporary. Subsequently integrins on leukocytes, especially CD11a/18, bind strongly to their ligand intercellular adhesion molecule-1 (ICAM-1) on vascular endothelial cells.

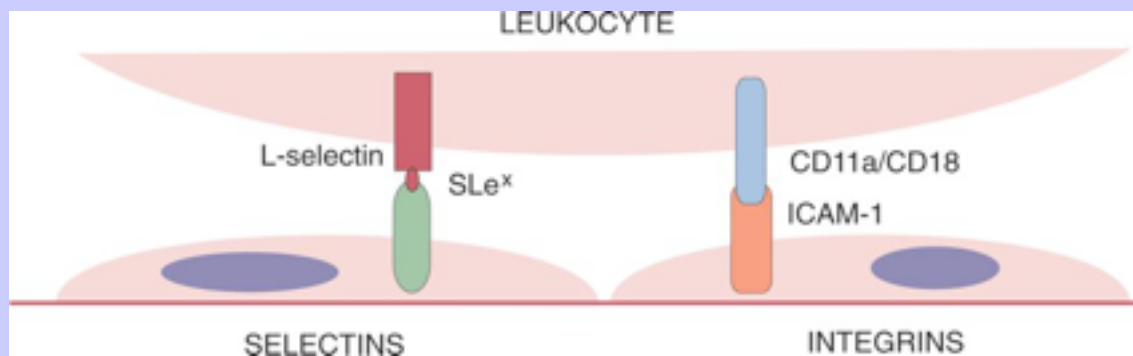
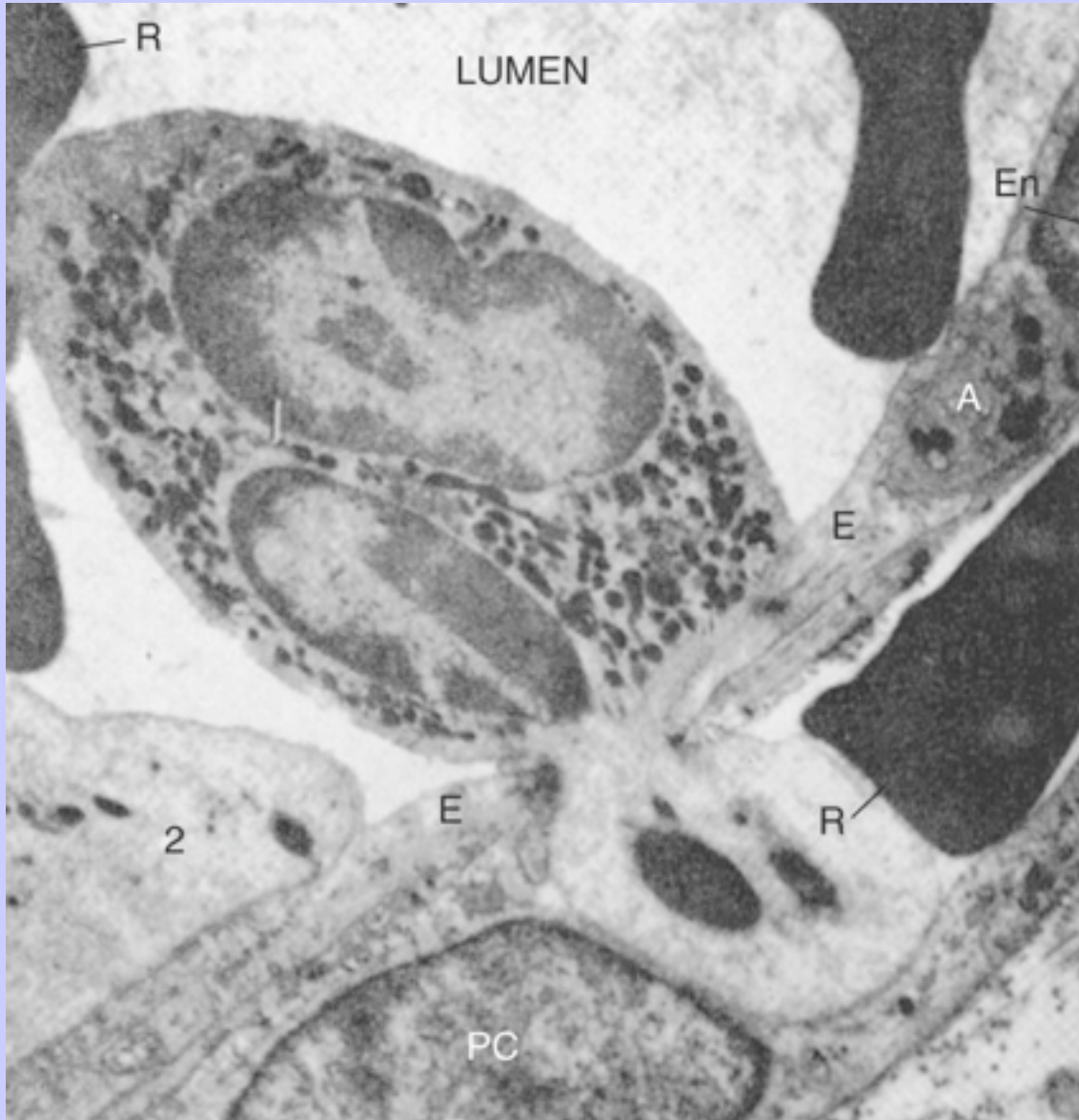


FIGURE 3-8 Inflamed venule of a rat. Cell 1 is a neutrophil pushing its way through a capillary wall to reach the surrounding tissues. R, Red blood cells; E, endothelium; PC, periendothelial cell; cells 2 and 3 are also neutrophils. (From Marchesi VT, Florey HW: Q J Exp Physiol 45:343, 1960.)



the neutrophils to bind to the endothelial cells even more strongly.

A third stage of increased leukocyte–endothelial cell adhesion takes several hours to develop and is mediated by cytokines and chemokines. Thus endothelial cells activated by interleukin-1 (IL-1), interleukin-23 (IL-23), or

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tumor necrosis factor- α (TNF- α) express E-selectin (CD62E) ([Figure 3-7](#)), which enhances neutrophil adhesiveness even further. IL-1 and IL-23 also induce the production of the chemokine CXCL8 by endothelial cells, and this attracts still more neutrophils. TNF- α stimulates endothelial cells to secrete IL-1. It also promotes vasodilatation, procoagulant activity, and thrombosis, and increases both expression of cell adherence proteins and the production of chemotactic molecules.

3.4.3

Integrins

Many cell surface proteins make cells stick together, but the most important of these are the integrins. There are several families of integrins. Each consists of paired protein chains (heterodimers) using a unique α chain linked to a common β chain. For example, three β_2 -integrins are found on neutrophils. The α chain, called CD11a, b, or c, is linked to a common β_2 chain (CD18). So these three integrins are called CD11a/CD18, CD11b/CD18, and CD11c/CD18. As described above, LFA-1 expressed by activated neutrophils binds to ICAM-1 expressed on capillary endothelial cells. CD11b/CD18 also binds leukocytes to endothelial cells and is a receptor for some components of the complement system (complement receptor 3 [CR3]) (see [Chapter 5](#)).

3.4.4

Emigration

After binding to blood vessel walls and coming to a complete stop, the neutrophils emigrate into the surrounding tissues under the influence of chemoattractants ([Figure 3-8](#)). The migrating neutrophils squeeze between the endothelial cells and the basement membrane. This process has been called diapedesis or transmigration. They then crawl towards any invading microbes. Since neutrophils are the most mobile of all the blood leukocytes, they are the first cells to arrive at the damaged tissues.

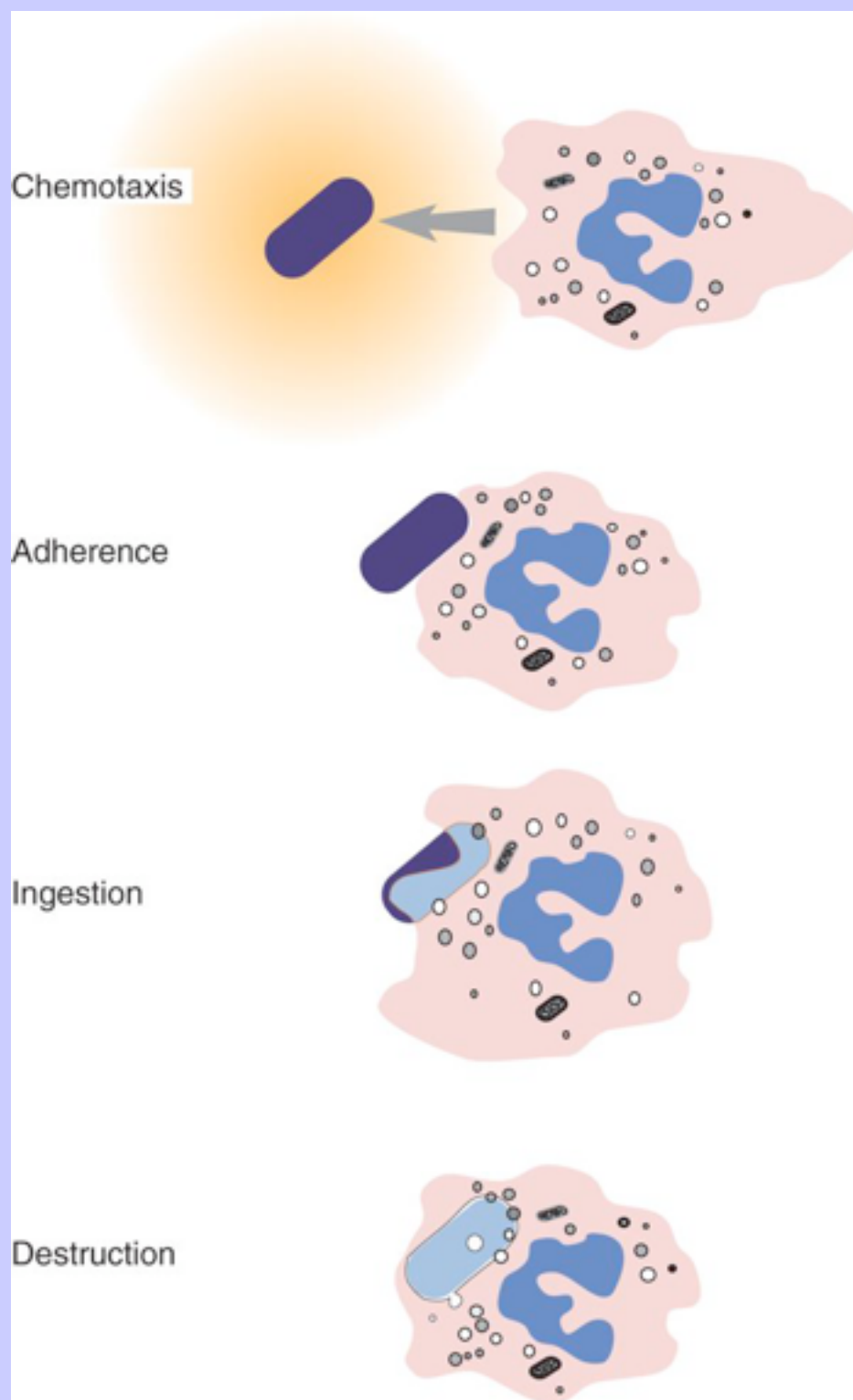
3.5

PHAGOCYTOSIS

Once they reach sites of microbial invasion, neutrophils eat and destroy foreign particles such as invading bacteria through phagocytosis. Although a continuous process, phagocytosis can be divided into discrete stages: activation, chemotaxis, adherence, ingestion, and destruction ([Figure 3-9](#)).

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FIGURE 3-9 Different stages in the process of phago-cytosis.



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3.5.1 Activation

Neutrophils attack and destroy invading organisms after they have become “activated.” Thus, when neutrophils receive the dual signal of integrin binding together with stimulation by TNF- α , CXCL8, or C5a, they secrete elastase, defensins, and oxidants. The elastase released promotes their adhesiveness. The oxidants activate tissue metalloproteases, which in turn cleave more TNF- α from macrophages. The TNF- α in turn attracts more neutrophils.

3.5.2 Chemotaxis

Neutrophils do not wander randomly but crawl directly towards invading organisms and damaged cells in a process called chemotaxis. Microbial invasion and the resulting tissue damage generate many different attractants. These include a peptide called C5a, generated by activation of complement (see [Chapter 5](#)); a peptide called fibrinopeptide B, derived from fibrinogen; and a peptide called azurocidin related to the defensins. Other chemoattractants include many different chemokines (see [Chapter 2](#)) and lipids such as leukotriene B₄. Invading bacteria release peptides with formylated methionine groups that are very attractive to the neutrophils of some mammals. Thus migrating neutrophils receive a multitude of signals attracting them to sites of invasion and tissue damage.

Not all animals have equally responsive neutrophils. For example, some cows with a specific genotype of the chemokine receptor CXCR2 show reduced neutrophil migration compared to cows with other genotypes. Cows with this specific genotype also show reduced expression of the integrin chains CD18 and CD11b and decreased resistance to mastitis (infections of the udder).

As chemotactic molecules diffuse from sites of a microbial invasion, they form a concentration gradient. When neutrophils detect these molecules, they crawl toward the area of highest concentration—the source of the material. The moving cells generate projections (lamellipodia) at their leading edge. Chemoattractant receptors are distributed over the neutrophil surface, but the formation of lamellipodia is driven by the higher concentration of attractants at the cell's leading edge.

3.5.3 Adherence and Opsonization

Once a neutrophil encounters a bacterium, it must “catch” it. This does not happen spontaneously, because both cells and bacteria suspended in body fluids usually have a negative charge (zeta potential) and so repel each other. The charge must be neutralized, which requires that the bacteria be coated with positively charged molecules. Molecules that coat bacteria in this way and so promote phagocytosis are called opsonins. This word is derived from the Greek word for “sauce,” implying perhaps that they make the bacterium “tastier” for the neutrophil. Examples of such charged molecules include innate molecules such as mannose-binding lectin and complement components and acquired molecules such as antibodies (see [Chapter 14](#)).

The surface of phagocytic cells is also adorned with many receptors that can recognize their ligands on the surface of infectious agents. These receptors may recognize a particle directly or recognize opsonins. Ingestion may or may not depend on opsonization. Thus neutrophils have some cell surface receptors such as mannose receptors or integrins that bind directly to bacteria.

Antibody receptor-mediated phagocytosis (or type I phagocytosis) is triggered by the binding of antibody-coated bacteria to antibody receptors on the neutrophil surface (Figure 3-10). CD32 is an example of such an antibody receptor. When antibody-coated microbes

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FIGURE 3-10 Opsonization of a bacterium by antibodies and complement. The combination of these ligands with their appropriate receptors triggers ingestion and the respiratory burst. The antibody receptor is called CD32, and the complement receptor is called CD35. Type 1 phagocytosis is mediated by antibodies through CD32. Type 2 phagocytosis is mediated by complement through CD35.

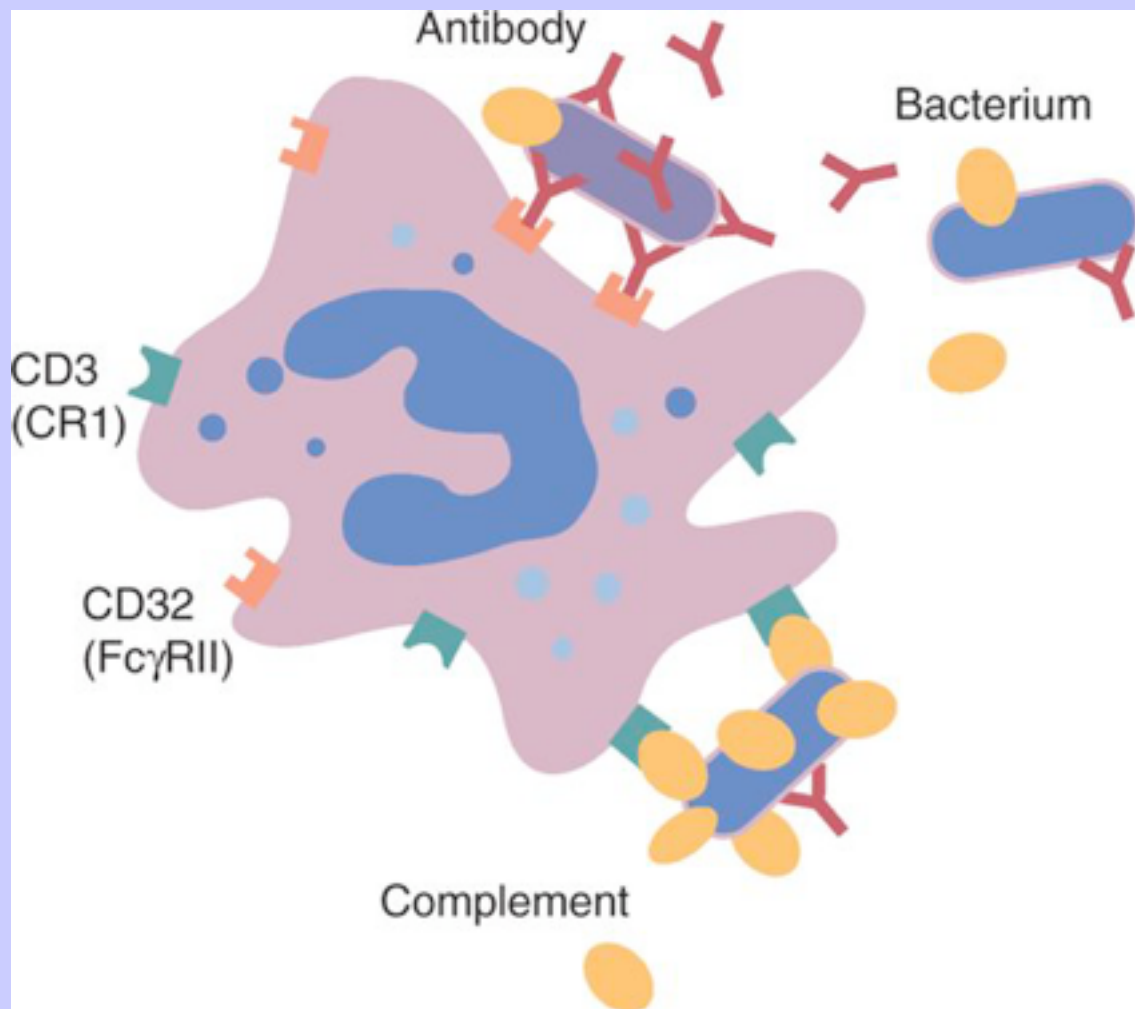
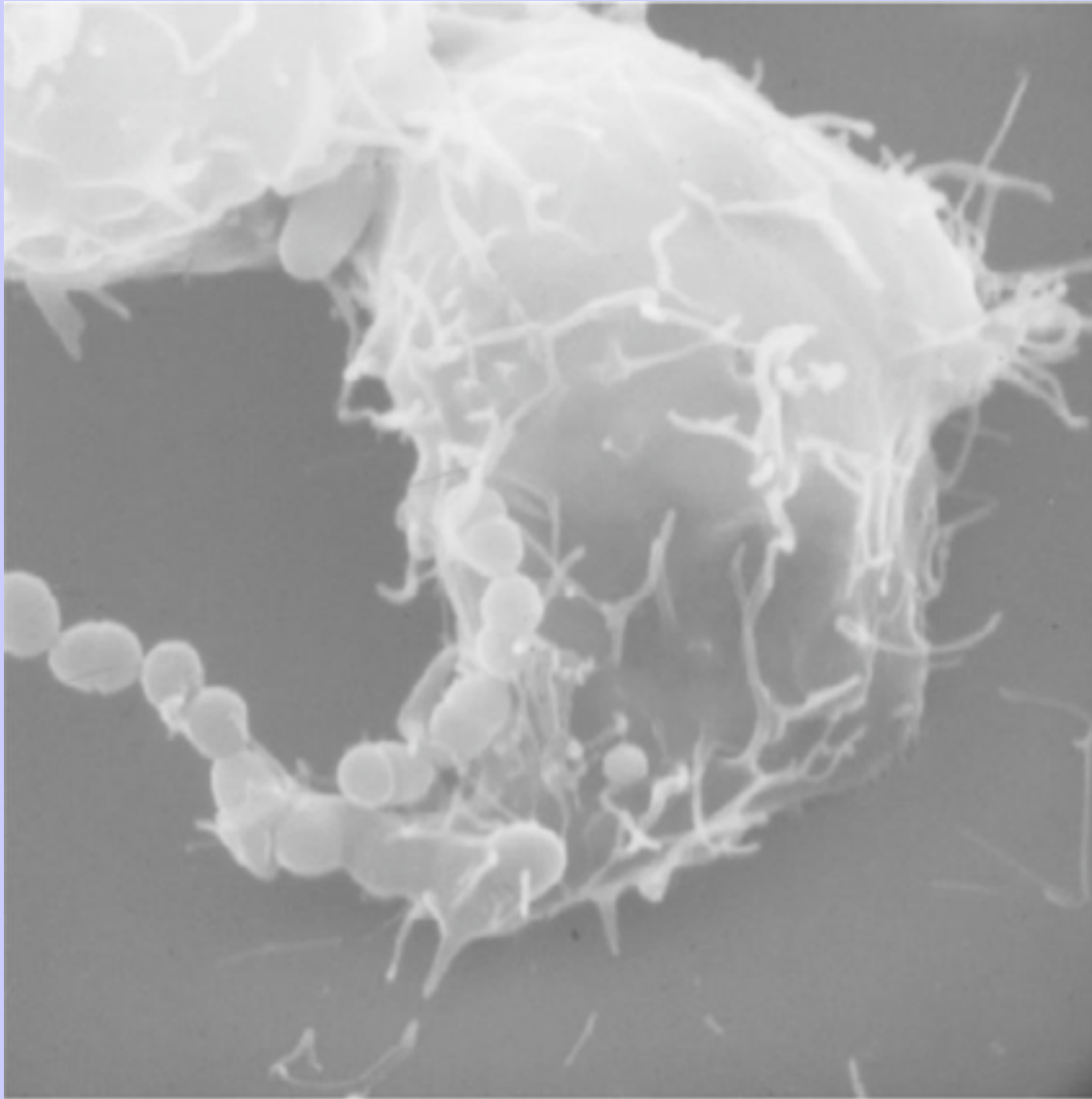


FIGURE 3-11 Scanning electron micrograph of a bovine milk neutrophil ingesting *Streptococcus agalactiae*. Note how a film of neutrophil cytoplasm appears to flow over the surface of the bacterium. Original magnification $\times 5000$.



bind to CD32, they trigger polymerization of F-actin. As a result, F-actin-rich lamellipodia extend from the cell to engulf the particle. The ligand of CD32 is a site on the Fc region of antibody molecules (see [Chapter 13](#)). CD32 is therefore an example of an Fc receptor (FcR). (Since there are several different Fc receptors, CD32 is classified as Fc γ RII.)

In complement-mediated phagocytosis (type II phagocytosis), particles sink into the neutrophil without lamellipodia formation, suggesting that the ingestion process is fundamentally different from the antibody-

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mediated process. CD35 (or complement receptor 1 [CR1]) is a receptor for the complement component C3b. CR1 is found not only on neutrophils but also on other granulocytes, monocytes, red cells, and B cells. Binding of C3b-coated particles to neutrophil CD35 leads to their attachment but may not necessarily trigger ingestion.

Antibodies, the major proteins of the acquired immune system, are by far the most effective opsonins. They coat bacteria, link them to receptors on phagocytic cells, and trigger their ingestion. However, as pointed out previously, antibodies are not produced until several days after the onset of an infection, and the body must therefore rely on innate opsonins for immediate protection.

Another important mechanism that promotes contact between bacteria and neutrophils is trapping. Normally bacteria are free to float away when they encounter a neutrophil suspended in blood plasma. If, however, a bacterium is lodged in tissues, or trapped between a neutrophil and another cell surface and thus prevented from floating away, it can be readily ingested. This process is called surface phagocytosis.

Although it has generally been accepted that neutrophils ingest bacteria before killing them, they can also trap and kill extracellular bacteria. After activation by IL-8 or LPS, neutrophil granule proteins and chromatin are released into the extracellular fluid, where together they form a network of extracellular fibers. Not only can these fibers physically capture bacteria, but they can also kill them and destroy their virulence factors. These neutrophil extracellular traps (NETS) are abundant at sites of acute inflammation and are found, for example, in mastitic milk.

3.5.4 Ingestion

As neutrophils crawl toward a chemotactic source, a lamellipod advances first, followed by the main portion of the cell. The cytoplasm of the neutrophil lamellipodia contains a filamentous network of actin and myosin whose state determines the fluidity of the cytoplasm. When a neutrophil meets a bacterium, its lamellipod flows over and around the organism and binding occurs between opsonins on the organism and receptors on the neutrophil surface ([Figure 3-11](#)).

Binding of these receptors enables a cuplike structure to cover the particle. The bacterium is eventually drawn into the cell; as it is engulfed, it becomes enclosed in a vacuole called a phagosome. The ease of this ingestion depends on the properties of the bacterial surface. Neutrophil cytoplasm readily flows over lipid surfaces so that hydrophobic bacteria, such as *Mycobacterium tuberculosis*, are readily ingested. In contrast, *Streptococcus pneumoniae*, a cause of pneumonia in humans, has a hydrophilic capsule. It is poorly phagocytosed unless made hydrophobic by opsonization. The progressive covering of a particle by the linkage of cell receptors with ligands on the particle has been likened to a zipper. An alternative process is called coiling phagocytosis. In this case a single lamellipod may wrap itself several times around the organism. This is associated with bacteria such as *Legionella pneumophila* and *Borrelia burgdorferi*.

3.5.5 Destruction

Destruction of the ingested bacterium occurs through two distinct processes. One, the respiratory burst, involves the generation of potent oxidants. The other involves release of lytic enzymes and antimicrobial peptides from intracellular granules.

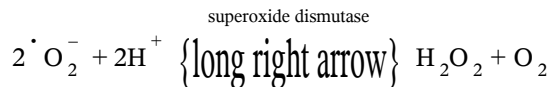
3.5.5.1

The Respiratory Burst

Within seconds of binding to a bacterium, neutrophils increase their oxygen consumption nearly 100-fold. This increase is a result of activation of a cell surface enzyme complex called NADPH oxidase (NOX). The components of the NOX complex are separated in resting cells. When a neutrophil binds cytokines such as TNF- α or is exposed to other inflammatory stimuli, the NOX complex is assembled ([Figure 3-12](#)). Once assembled, the activated NOX converts NADPH (the reduced form of NADP, nicotinamide adenine dinucleotide phosphate) to NADP⁺ with the release of electrons. A molecule of oxygen accepts a donated electron, resulting in the generation of a superoxide anion (the dot in $\cdot\text{O}_2^-$ denotes the presence of an unpaired electron):

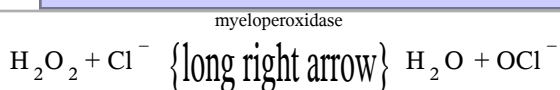
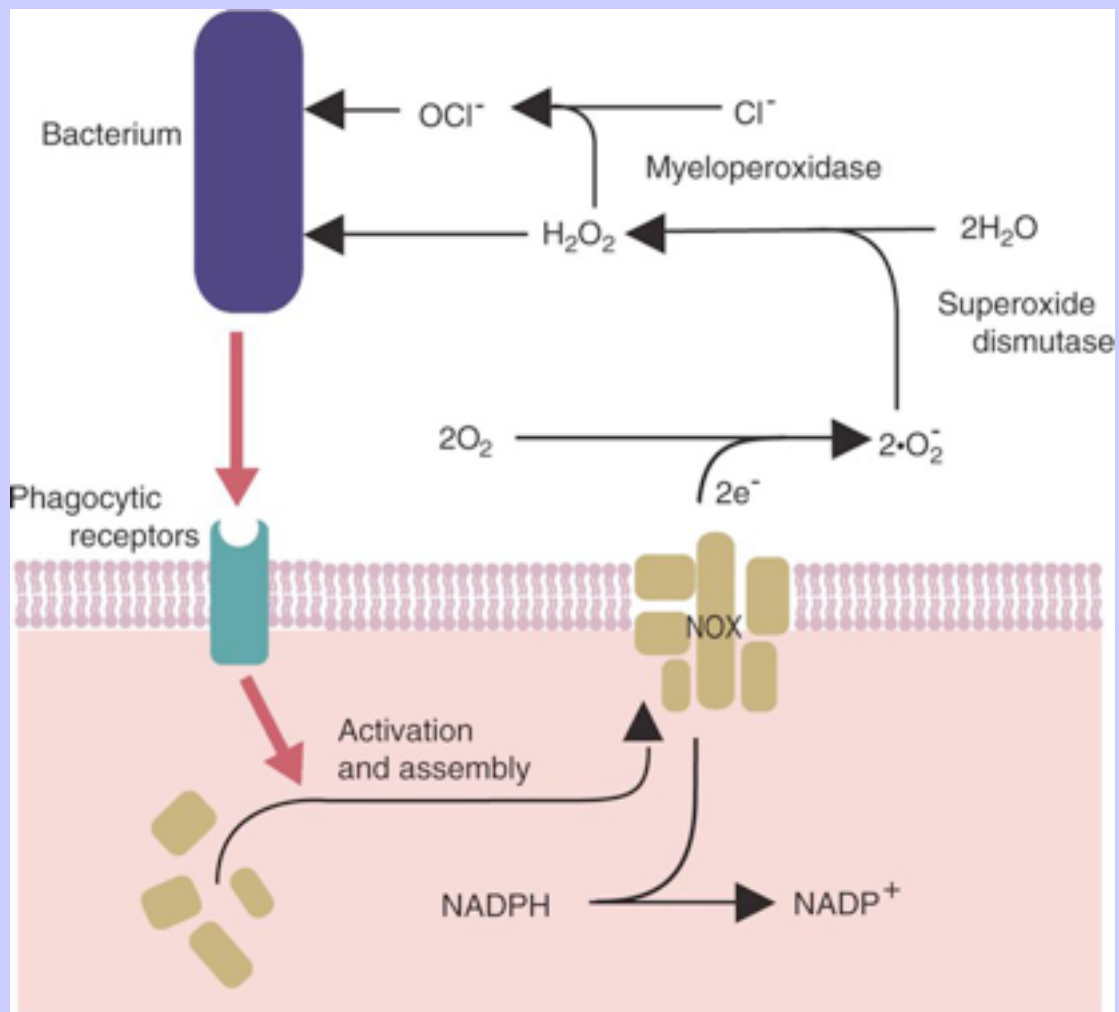


The NADP⁺ accelerates the hexose monophosphate shunt, a metabolic pathway that converts glucose to a pentose and CO₂ and releases energy for use by the cell. The two molecules of $\cdot\text{O}_2^-$ interact spontaneously (dismutate) to generate one molecule of H₂O₂ under the influence of the enzyme superoxide dismutase:



Because this reaction occurs so rapidly, superoxide anion does not accumulate but H₂O₂ does. The hydrogen peroxide is converted to bactericidal compounds through the action of myeloperoxidase, the most significant respiratory burst enzyme in neutrophil granules. Myeloperoxidase catalyzes the reaction between hydrogen peroxide and intracellular halide ions (Cl⁻, Br⁻, I⁻, or SCN⁻) to produce hypohalides:

FIGURE 3-12 The major features of the respiratory burst pathway in neutrophils. The process is triggered by cytokines such as $\text{TNF-}\alpha$ or by binding of opsonized bacteria to phagocytic receptors. It results in the assembly of the multicomponent enzyme NADPH oxidase (NOX) in the membrane of the phagosome. Once assembled, NOX catalyses the generation of bactericidal products such as hydrogen peroxide (H_2O_2) and hypochloride ions (OCl^-).



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Plasma Cl^- is probably used at most inflammatory sites except in milk and saliva, where SCN^- is also employed. OCI^- is the major product of neutrophil oxidative metabolism. Because of its reactivity, OCI^- does not accumulate in biological systems but disappears in multiple reactions. As long as H_2O_2 is supplied, and neutrophils can generate H_2O_2 for up to 3 hours after triggering, myeloperoxidase will use plasma Cl^- to generate OCI^- . OCI^- kills bacteria by oxidizing their proteins and lipids and enhances the bactericidal activities of the lysosomal enzymes. (Remember that HOCl is the active ingredient of household bleach and is commonly used to prevent bacterial growth in swimming pools.) There are minor quantitative differences in neutrophil activity between the domestic species, especially in the intensity of the respiratory burst. For example, sheep neutrophils appear to produce less superoxide than human or bovine neutrophils. Neutrophils also have safety mechanisms to detoxify oxidants and minimize collateral damage. Thus they contain large amounts of glutathione, which reduces the oxidants. Redox-active metals such as iron can be bound to lactoferrin to minimize OH formation, and antioxidants such as ascorbate or vitamin E interrupt these reactions.

3.6 ANTIMICROBIAL MOLECULES

3.6.1 Lytic Enzymes

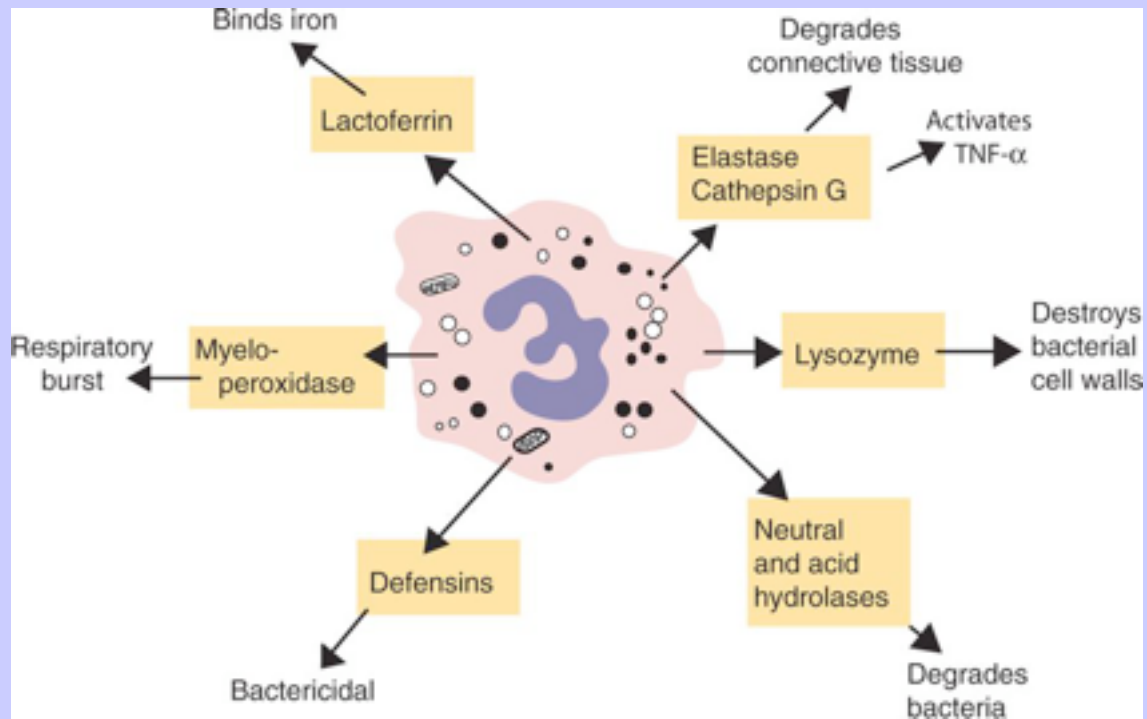
While many bacteria are killed by oxidation, the phagosomes continue to mature. They progressively acidify their contents, reducing their pH to a level optimal for granule proteases.

Once a bacterium is attached to the neutrophil membrane, the granules (or lysosomes) migrate through the cytoplasm, fuse with the maturing phagosome, and release their enzymes. (The complete vacuole is then called a phagolysosome.) The rise in ionic strength within phagosomes releases enzymes such as elastase and cathepsin G from their sulfated proteoglycan matrix ([Figure 3-13](#)). Other lysosomal enzymes include lysozyme, proteases, acid hydrolases, and myeloperoxidase. The enzymes that accumulate in phagosomes can digest bacterial walls and kill most microorganisms, but, as might be expected, variations in susceptibility are observed. Gram-positive bacteria susceptible to lysozyme are rapidly destroyed. Gram-negative bacteria such as *Escherichia coli* survive somewhat longer, since their outer wall is relatively resistant to digestion. Lactoferrin, by binding iron, may deprive bacteria of this essential nutrient and so limit bacterial growth. Some organisms such as *Brucella abortus* and *Listeria monocytogenes* can interfere with phagosomal maturation in such a way that they do not come into contact with the lysosomal enzymes and can therefore grow inside phagocytic cells. Neutrophil enzymes released into tissues cleave membrane-bound $\text{TNF-}\alpha$ from macrophages. The $\text{TNF-}\alpha$ attracts and activates yet more neutrophils.

3.6.2 Peptides

Antimicrobial peptides are widely distributed throughout the plant and animal kingdoms, and more than 800 have been identified to date. Although structurally diverse, these peptides have a net cationic charge due to the presence of multiple arginine and lysine residues and the ability to form amphipathic structures;

FIGURE 3-13 Some of the enzymes and other antibacterial molecules found in the diverse cytoplasmic granules of neutrophils.



that is, they have both hydrophobic and hydrophilic regions. The hydrophobic regions can insert themselves into the lipid-rich membranes of bacteria, whereas the other regions can form channel-like pores or simply cover the membrane. This results in membrane disruption and microbial death.

The cationic antimicrobial peptides can kill most species of bacteria as well as some fungi, protozoa, enveloped viruses, and tumor cells. The fact that they kill microorganisms rather than host cells is thought to be due to their interactions with microbial phospholipids, LPS, or teichoic acids.

Antimicrobial peptides are concentrated in sites where microbes are most likely to be encountered. These include intracellular locations such as within neutrophils and macrophages (see [Chapter 4](#)) and within lymphoid organs (see [Chapter 10](#)). Epithelial cells of the skin and respiratory, alimentary, and genitourinary tracts also synthesize antimicrobial peptides.

The defensins are typical antimicrobial peptides. Defensins contain 28 to 42 amino acids arranged in a β -sheet that contains three or four disulfide bonds. More than 50 different mammalian defensins have been identified. The vertebrate defensins are classified as α , β , or θ defensins based on their origin and on the number and position of these disulfide bonds. The α defensins account for about 15% of the total protein in neutrophil granules. In cattle at least 13 different α defensins are produced by neutrophils alone. They are also found in the granules of Paneth cells in the small intestine; β defensins are expressed in many different tissues including the epithelial cells that line the airways, skin, salivary gland, and urinary system. Theta defensin is a circular peptide that is found only in primate neutrophils. Defensins can be produced at a constant rate (constitutively) or in response to microbial infection. Some defensins attract monocytes, immature dendritic cells, and T cells. All defensins identified so far

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can kill or inactivate some bacteria, fungi, or enveloped viruses. Some defensins can also neutralize microbial toxins such as the toxins of *Bacillus anthracis*, *Corynebacterium diphtheriae*, and staphylokinase from *Staphylococcus aureus*.

Although present in normal tissues, defensin concentrations increase in response to infections. For example, calves infected with *Cryptosporidium parvum* or *Mycobacterium paratuberculosis* show a significant increase in cryptdin production. *Mannheimia hemolytica* infection in bovine lungs induces increased defensin expression in airway epithelium.

The second major classes of antibacterial peptides in neutrophil granules are the cathelicidins. These are peptides ranging from 12 to 80 amino acids in size with a broad range of antibacterial activity. They are all stored within cells in an inactive form attached to a conserved precursor N-terminal peptide of about 100 amino acids and released following cleavage of the precursor molecule. They are named using acronyms or amino acid symbols followed by the number of amino acids they contain. Humans and mice have only one cathelicidin gene while the pig, cow, and horse have multiple cathelicidin genes. Porcine cathelicidin PR-39 has been shown to promote wound repair, angiogenesis, and neutrophil chemotaxis. The bovine cathelicidin BMAP-28 induces apoptosis in some cells and may serve to get rid of unwanted cells. Many of these peptides have been given their own specific names such as protegrins, novispirin, and ovispirin.

Other antibacterial peptides include the serprocidins and the granulysins. Serprocidins are antimicrobial serine proteases found in the primary granules of neutrophils. Granulysins are peptides produced by cytotoxic T cells and natural killer cells (see [Chapters 16](#) and [30](#)). In addition to their antibacterial functions, granulysins are chemoattractants and activate macrophages. Two other important antibacterial proteins include bactericidal permeability-increasing protein (BPI) and calprotectin. BPI is a major constituent of the primary granules of human and rabbit neutrophils. It kills Gram-negative bacteria by binding to LPSs and damaging their inner membrane. Calprotectin is found in neutrophils, monocytes, macrophages, and epidermal cells. It forms about 60% of neutrophil cytoplasmic protein and is released in large amounts into blood and tissue fluid in inflammation.

3.6.3

Lysozyme

The enzyme lysozyme cleaves the bond between N-acetyl muraminic acid and N-acetyl glucosamine and so destroys the peptidoglycans of Gram-positive bacteria. Lysozyme is found in all body fluids except cerebrospinal fluid and urine. It is absent from bovine neutrophils and tears. It is found in high concentrations in tears of other mammals and in egg white. Although many of the bacteria killed by lysozyme are nonpathogenic, it might reasonably be pointed out that this susceptibility could account for their lack of pathogenicity. Lysozyme is found in high concentrations in some neutrophil granules and so accumulates in areas of acute inflammation, including sites of bacterial invasion. Lysozyme is also a potent innate opsonin, binding to bacterial surfaces and so facilitating phagocytosis in the absence of specific antibodies and under conditions where its enzyme activity is ineffective.

3.6.4

Lectins

Lectins are proteins that bind carbohydrates. Given that carbohydrates are major components of bacterial cell walls, lectins often bind to bacteria. Mammalian lectins are classified into P-, S-, and C-type lectins ([Figure 3-14](#)).

The pentraxins are P-type lectins. They include two important molecules: C-reactive protein (CRP) and serum amyloid P (SAP). These are called acute-phase proteins because their blood levels climb greatly during infections

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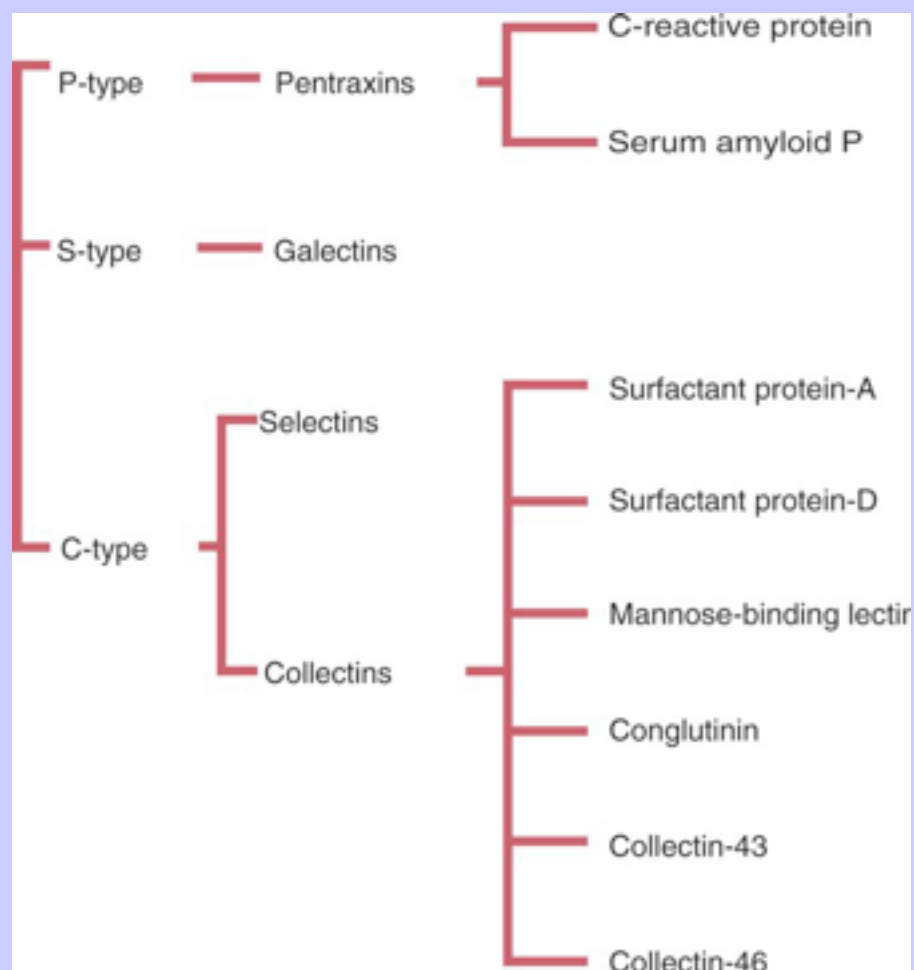
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or following trauma. Pentraxins have multiple biological functions, including activation of the complement system and stimulation of leukocytes. Pentraxin molecules consist of five protein subunits arranged in a ring. Pentraxins bind to carbohydrates such as bacterial LPS in a calcium-dependent manner. Both CRP and SAP can activate the classical complement pathway by interacting with C1q (see [Chapter 5](#)). They also interact with neutrophils, monocytes/macrophages, and natural killer cells and augment their activities. For example, CRP not only binds to phosphocholine, a molecule found in all cell membranes, but its major receptor on leukocytes is FcγRII (CD32). It can bind to invading organisms and promote their phagocytosis. SAP binds to galactose polymers and glycosaminoglycans.

The galectins are S-type lectins. Their name derives from their specificity for galactosides. They play a role in inflammation by mediating the binding of leukocytes to extracellular matrix.

FIGURE 3-14 Carbohydrate-binding proteins called lectins play important roles in the defense of the body. They can bind to microbial carbohydrates and then serve as opsonins or activators of other innate defenses. They are diverse, and they belong to multiple protein families.



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C-type lectins require calcium to bind to carbohydrates. They include the selectins involved in leukocyte adherence to blood vessel walls and the collectins that are key components of the innate immune system. Each end of a collectin molecule has a distinct function: the C-terminal domain binds to bacterial carbohydrates whereas the N-terminal domain interacts with cells and complement components, thereby exerting the biological effect. Their ability to recognize foreign carbohydrates makes collectins a part of the innate host defense system.

The most important lectin in the innate immune system is mannose-binding lectin (MBL), a collectin found in serum. MBL has multiple carbohydrate-binding sites that bind oligosaccharides, such as N-acetylglucosamine, mannose, glucose, galactose, and N-acetylgalactosamine. The binding is relatively weak, but multiple binding sites give a high functional activity. Most of the ligands of MBL are present at high levels on microbial surfaces. As a result, MBL binds very strongly to bacteria such as *Salmonella enterica*, *L. monocytogenes*, *Haemophilus influenzae*, and *Neisseria meningitidis*. It binds to *Streptococcus* and *E. coli* with moderate affinity, but it does not bind to encapsulated *N. meningitidis*, *H. influenzae*, or *Streptococcus agalactiae*. MBL binds strongly to yeasts such as *Candida albicans* and *Cryptococcus neoformans*. It can bind viruses such as human immunodeficiency virus and influenza A as well as the protozoan parasite *Leishmania*. MBL plays an important role in activating the complement system (see [Chapter 5](#)). There are two forms of mannose binding lectin in the pig called MBL-A and MBL-C. These can bind to *Actinobacillus suis* and *Haemophilus parasuis*. Some European pig breeds may express very low levels of MBL-C and hence suffer from increased disease susceptibility.

Collectins such as MBL can bind to leukocytes, platelets, endothelial cells, and fibroblasts. Bacteria coated by MBL are readily ingested by phagocytic cells through interaction with surface receptors. The collectins are especially important in young animals whose acquired immune system is not capable of mounting an efficient response. As a result, a congenital deficiency of MBL makes children highly susceptible to infections. Six different collectins (conglutinin, MBL, pulmonary surfactant proteins [SP-A, SP-D], and collectin-46 [CL-46] and CL-43) have been identified in mammals. However, conglutinin, CL-46, and CL-43 have only been identified in bovidae.

3.6.5

Iron-Binding Proteins

One of the most important innate factors that determines the success or failure of bacterial invasion is the availability of iron. Many pathogenic bacteria, such as *S. aureus*, *E. coli*, *B. anthracis*, *Pasteurella multocida*, and *M. tuberculosis*, require large amounts of iron for growth given that iron is the key catalytic site for many enzymes. However, animal hosts also require iron to survive. As a result, both microbe and host compete for the same metal. Iron concentrations within animal tissues are normally very low. Mammalian blood has just 10^{-26} M free iron since almost all available iron is bound to the iron-binding protein transferrin. Thus one effective defensive strategy is to remove as much iron as possible from sites of bacterial invasion. Within the body, iron is associated with several iron-binding proteins including transferrin, lactoferrin, siderocalin, haptoglobin, and ferritin. When bacteria invade the body, intestinal iron absorption ceases. Liver cells are stimulated to secrete transferrin and haptoglobin, and there is increased incorporation of iron into the liver. This effectively reduces the availability of iron still further. A similar situation occurs in the mammary gland when, in response to bacterial invasion, milk neutrophils release their stores of lactoferrin. The lactoferrin binds free iron and makes it unavailable to the bacteria. In spite of the reduced availability of iron, some bacteria, such as *M. tuberculosis*, *B. anthracis*, and *E. coli*, can successfully invade the body because they produce potent iron-binding proteins (siderophores) that can remove the iron from transferrin or lactoferrin. In effect, the body and the bacteria engage in a “tug-of-war” for iron molecules. Mycobacteria use their siderophore carboxymycobactin to strip iron from mammalian ferritin. The outcome of this competition may determine the outcome of the infection. When serum

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iron levels are elevated, as occurs following red cell destruction, animals may become more susceptible to bacterial infections.

Mammals can also capture iron by stealing bacterial siderophores. Thus during bacterial infections the mammalian liver, spleen, and macrophages synthesize a protein called lipocalin 2. Lipocalin 2 (also called siderocalin) binds the bacterial siderophore enterochelin with very high affinity. Lipocalin 2 is essential for limiting the growth of enterochelin-producing bacteria such as *E. coli* but does not affect the growth of bacteria that use other methods of acquiring iron.

3.6.6

Complement

The complement system consists of a complex mixture of enzymes, regulatory proteins, and receptors that plays a major role in innate antimicrobial immunity. This system, described in detail in [Chapter 5](#), can be activated simply by exposing microbial cell walls to serum proteins. It can also be activated by MBL bound to microbial walls, or even by antibodies on microbial cell walls. Once activated, complement components, especially C3 (the third component), bind irreversibly to bacteria and initiate bacterial killing or phagocytosis.

3.6.7

Cytokines

Under the influence of bacterial products such as LPS, neutrophils can secrete many different cytokines such as IL-1 α , IL-1 β , IL-1RA, TNF- α , IL-6, CXCL8 (IL-8), IL-10, and transforming growth factor- β . Although they produce only small quantities of these cytokines, neutrophils invade inflammatory sites in large numbers, so their total contribution may be significant.

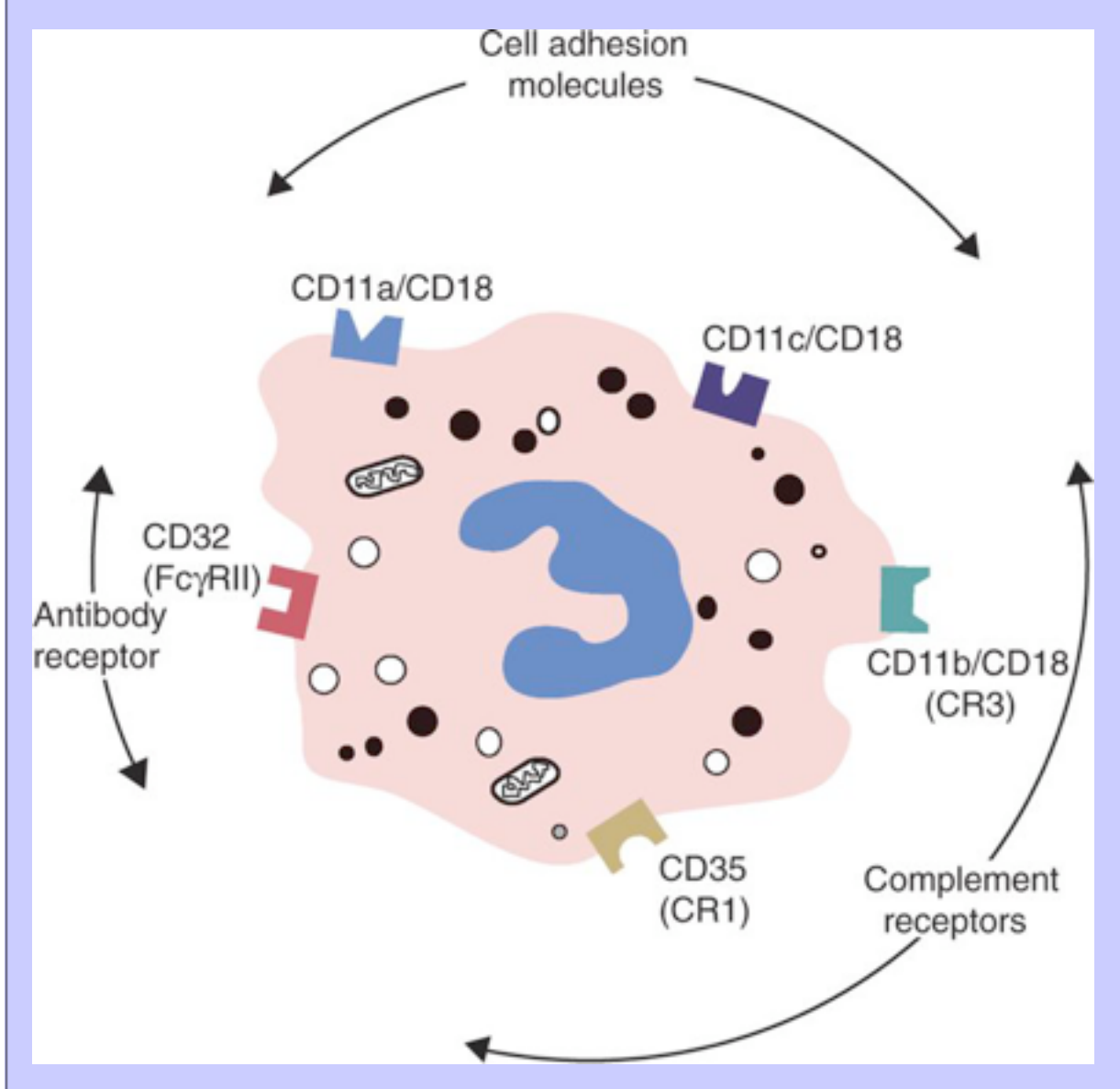
3.7

SURFACE RECEPTORS

Cells must interact with many molecules in their environment. To this end they express many different cell surface receptors. As mentioned in [Chapter 2](#), cell surface glycoproteins are classified by the cluster of differentiation (CD) system. Neutrophils carry many different CD molecules on their surface ([Figure 3-15](#)). The most relevant of these proteins are the receptors for opsonins and those that mediate neutrophil attachment to blood vessel walls. Other neutrophil surface molecules include receptors for inflammatory mediators such as leukotrienes, complement components such as C5a, chemokines, and cytokines.

A recent finding that tends to confuse rather than clarify cell identification is the fact that cells may exchange fragments of surface membrane together

FIGURE 3-15 Some of the major surface receptors on neutrophils and their functions.



with their receptors. For example, integral membrane proteins can be rapidly transferred to bovine neutrophils from a variety of apoptotic and necrotic cells. These include major histocompatibility complex class II molecules and CD3 from macrophages and T cells. This transfer is mediated by the fusion of membrane fragments or microvesicles to the neutrophil membrane and not by phagocytosis of cell fragments. These studies not only complicate studies on neutrophil cell membrane phenotypes, but they may well have implications for neutrophil functions.

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3.8 FATE

Neutrophils have a limited reserve of energy that cannot be replenished. They are therefore active immediately after being released from the bone marrow but are rapidly exhausted and can undertake only a limited number of phagocytic events. The vast majority of neutrophils survive for only a few days. Thus they may be considered a first line of defense, moving rapidly toward invading organisms and destroying them promptly but being incapable of sustained effort. The second line of defense is the mononuclear phagocyte system.

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4 CHAPTER 4 Macrophages and the Later Stages of Inflammation

4.1 KEY POINTS

- Macrophages move into sites of inflammation after neutrophils. They ingest and kill surviving microbial invaders.
- Macrophages ingest dead and dying neutrophils and so limit the damage caused by escaping neutrophil enzymes.
- Macrophages generate the powerful oxidizing agent nitric oxide.
- Macrophages effectively remove foreign particles from the bloodstream and the respiratory tract.
- Macrophages are responsible for beginning the healing process in damaged tissues.
- Cytokines secreted by sentinel cells cause a fever and are responsible for the behavioral changes that we call sickness.
- Excessive production of these cytokines (a cytokine storm) can lead to lethal shock syndromes.
- Excessive, chronic release of inflammatory cytokines can cause deposition of insoluble proteins called amyloid in tissues.

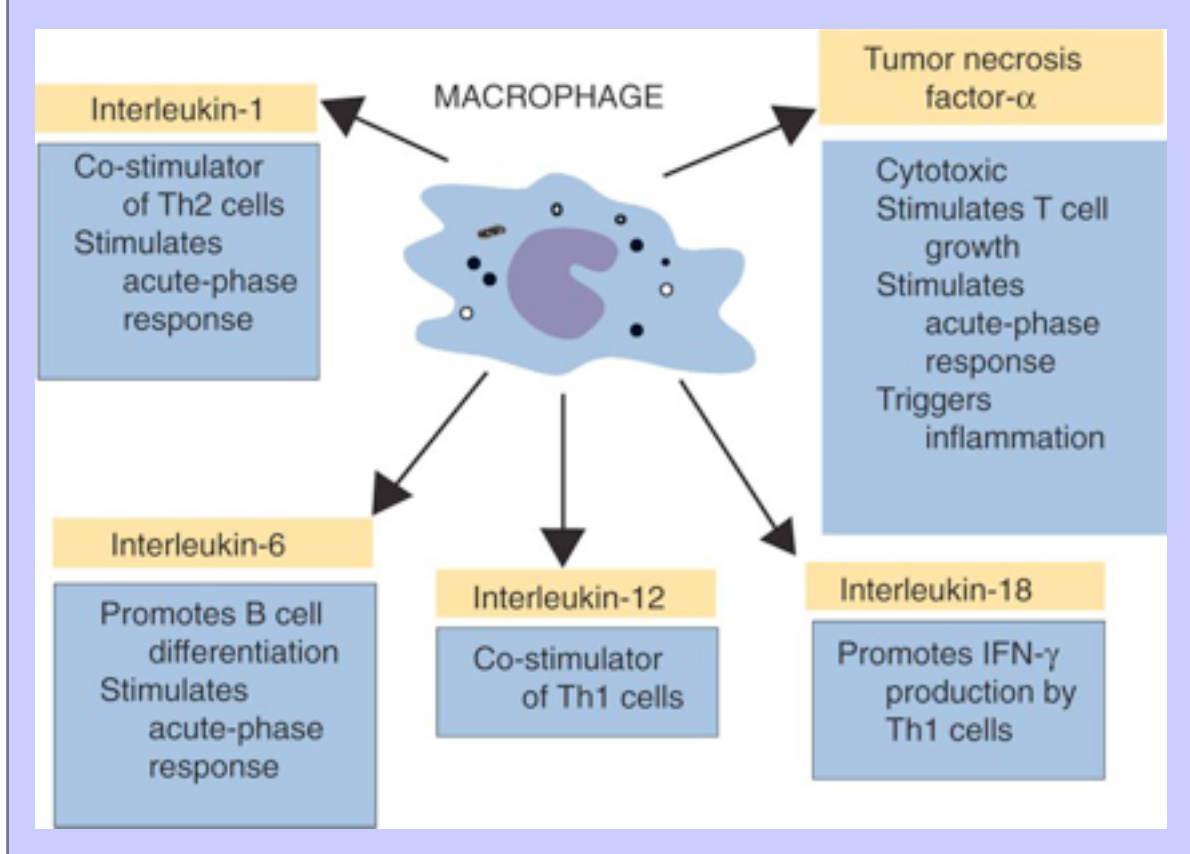
Although neutrophils act as a first line of defense, mobilizing rapidly and eating and killing invading microorganisms with enthusiasm, they cannot by themselves ensure that all invaders are killed. The body therefore employs a “backup” system employing phagocytic cells called monocytes (when in the bloodstream) and macrophages (when in tissues). Macrophages differ from neutrophils in their speed of response, which is slower; in their antimicrobial abilities, which are greater; and in their ability to stimulate acquired immune responses. Unlike neutrophils, which are specialized for the single task of killing invading organisms, macrophages have diverse functions. These include not only triggering inflammation by acting as sentinel cells, but also cleaning up the mess afterward.

4.2 MACROPHAGE FUNCTIONS

4.2.1 Sensors of Invasion

As described in [Chapter 2](#), macrophages possess both toll-like receptors (TLRs) and nucleotide-binding oligomerization domain–like receptors and so can detect invading bacteria and viruses. They respond by producing cytokines, the most important of which are interleukin-1 (IL-1), IL-12, IL-23, and tumor necrosis factor- α (TNF- α) ([Figure 4-1](#)).

FIGURE 4-1 The cytokines secreted by macrophages and their functions.



4.2.2

Phagocytosis

Monocytes bind to vascular endothelial cells in a manner similar to neutrophils. Thus cell rolling is triggered by selectin binding, and the cells are brought to a gradual halt by monocyte integrins binding ligands on blood vessel walls. Using β_2 integrins, the monocytes bind to endothelial cell intercellular adhesion molecule-1 and emigrate through the vessel walls. Within tissues these cells are called macrophages. Several hours after neutrophils have entered an inflammatory site, the macrophages begin to arrive.

Macrophages are attracted not only by bacterial products and complement components such as C5a but also by alarmins released from damaged cells and tissues. Defensins and other peptides from neutrophils attract monocytes and macrophages. Activated neutrophils and endothelial cells produce monocyte chemoattractant protein-1 (CCL2) under the influence of IL-6. Neutrophils are thus the martyrs of the immune system: They reach and attack foreign material first, and in dying they attract macrophages to the site of invasion. Phagocytosis by macrophages is similar to the process in neutrophils. Macrophages destroy bacteria by both oxidative and nonoxidative mechanisms. In contrast to neutrophils, however, macrophages can undertake sustained, repeated phagocytic activity. In addition, macrophages secrete collagenases and elastases that destroy connective tissue. They also release plasminogen activator that generates plasmin, another potent protease. Thus macrophages can “soften up” the local connective tissue matrix and so permit more effective penetration of the damaged tissue.

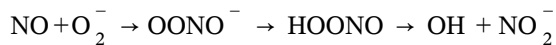
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Macrophages can phagocytose both apoptotic neutrophils and their granules. The contents of these neutrophil granules are not always destroyed but may be carried to endosomes, where they can inhibit the growth of bacteria such as *Mycobacteria*. Thus neutrophils can enhance the effectiveness of macrophages in host defense.

4.2.2.1

Generation of Nitric Oxide

In some mammals, especially rodents, cattle, sheep, and horses (but not in humans, pigs, goats, or rabbits), microbial products trigger macrophages to synthesize inducible nitric oxide synthase (NOS2). This enzyme uses NADPH and oxygen to act on L-arginine to produce large amounts of nitric oxide (nitrogen monoxide, NO) and citrulline ([Figure 4-2](#)). Although nitric oxide itself is not highly toxic, it can react with superoxide anion to produce highly reactive and toxic oxidants such as peroxynitrite and nitrogen dioxide radical.



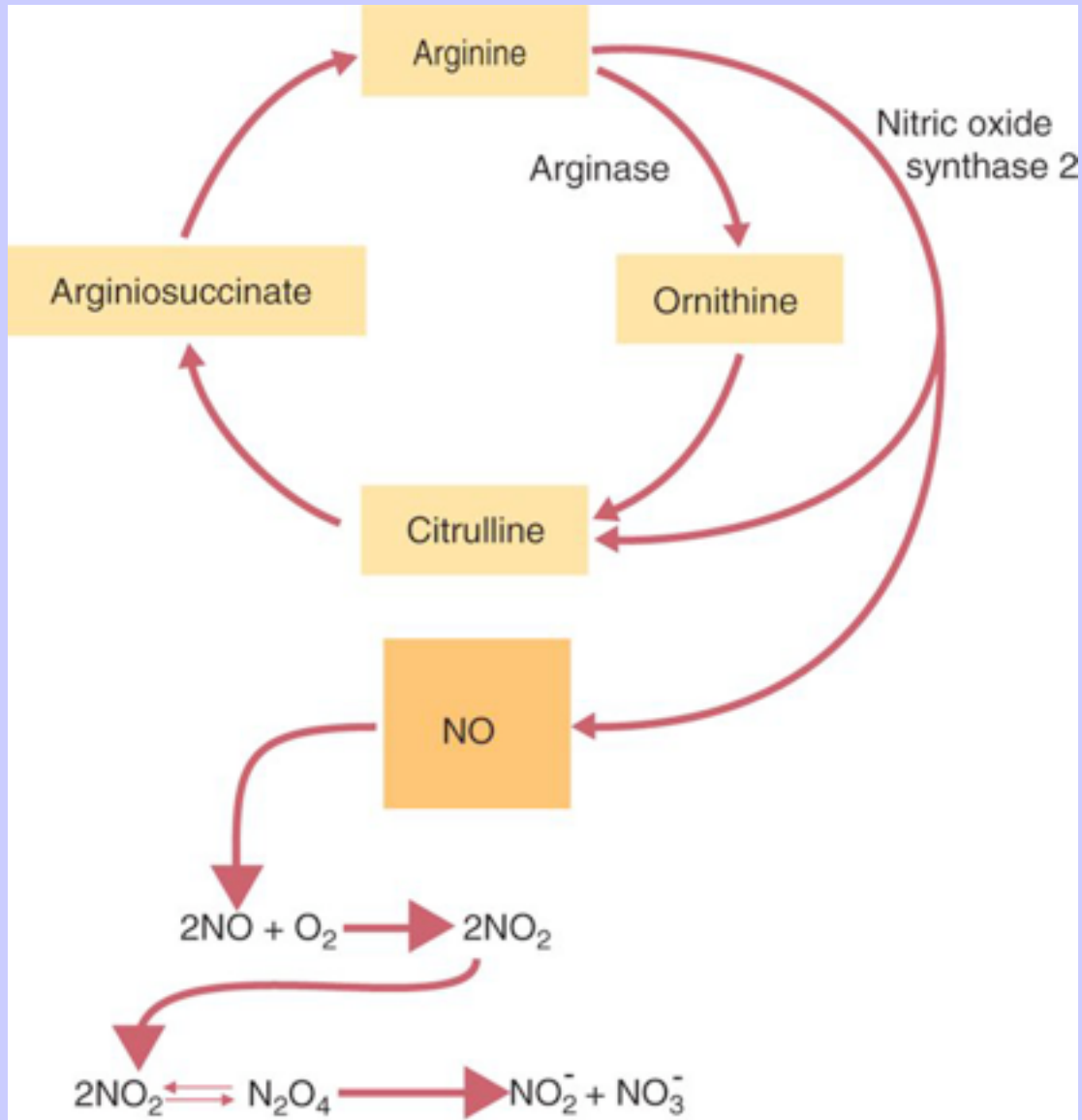
Nitric oxide Peroxynitrite anion Nitrogen dioxide radical

Not all macrophages generate nitric oxide. Those that do are called M1 cells, and their primary function is host defense. The sustained production of NO permits M1 macrophages to kill bacteria, fungi, protozoa, some helminths, and tumor cells very efficiently. Nitric oxide binds to metal-containing enzymes such as ribonucleotide reductase and impedes DNA synthesis. It also blocks mitochondrial heme-containing respiratory enzymes.

A second population of macrophages, called M2 cells, converts arginine to ornithine using the enzyme arginase and does not produce NO. These two macrophage populations play different roles in defending the body. M1 cells defend against microbial invaders and

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FIGURE 4-2 The two pathways of arginine metabolism in macrophages. The production of nitric oxide (NO) through the use of nitric oxide synthase 2 is a major antimicrobial pathway and the key feature of M1 macrophages. The use of arginase to produce ornithine, however, reduces the antimicrobial activities of M2 cells.



produce proinflammatory cytokines. M2 cells have opposite effects: They reduce inflammation and produce cytokines that suppress immune responses. M1 cells are produced early in the inflammatory process when inflammation is required. M2 cells, on the other hand, appear late in the process when healing is required. M2 cells thus promote blood vessel formation, tissue remodeling, and tissue repair.

4.2.2.1.1

Box 4-1 Genes That Control Innate Immunity

Innate resistance to mycobacteria *Brucella*, *Leishmania*, and *Salmonella enterica typhimurium* is controlled by a gene called natural resistance-associated macrophage protein (Nramp), which has been identified in humans, dogs, mice, sheep, bison, red deer, cattle, and chickens. Nramp codes for an ion transporter protein in macrophages called natural resistance-associated transporter protein (Nramp1). After phagocytosis, Nramp1 is acquired by the phagosomal membrane. It then acts to pump divalent metals out of the phagosome and so inhibits the growth of intracellular parasites by depriving them of metal ions. Cattle with the resistant allele effectively activate their macrophages and so control the in vitro growth of *Brucella abortus*. The difference between the resistant and susceptible alleles appears to be associated with a single nucleotide substitution in the Nramp gene.

4.2.3

Activation

Although macrophages are effective phagocytes, their activities can be enhanced by innate mechanisms. Activation triggers include the ligands for TLRs such as lipopolysaccharides, CpG DNA, microbial carbohydrates, and heat shock proteins as well as alarmins. Different levels of activation are recognized, depending on the triggering agent: some bacteria, such as *Mycobacterium tuberculosis*, are better able to activate macrophages than others. Thus when macrophages first move into inflamed tissues, they produce more lysosomal enzymes, increase phagocytic activity, increase the expression of antibody and complement receptors, and secrete more proteases ([Figure 4-3](#)). The cytokines produced by these M1 macrophages, especially TNF- α and IL-12, activate a population of lymphocytes called natural killer (NK) cells. The NK cells in turn secrete the cytokine interferon- γ (IFN- γ), which activates macrophages still further. IFN- γ upregulates many different genes, especially the gene for inducible nitric oxide synthase (NOS2). Thus the NOS2 gene can be upregulated 400-fold by a combination of IFN- γ and mycobacteria. As a result of increased NO production, M1 cells become very potent killers of bacteria ([Box 4-1](#)).

4.2.4

Receptors

Macrophages have many surface receptors, which may differ between subpopulations ([Figure 4-4](#)). In addition to the TLRs, they also possess receptors for antibodies. For example, CD64 is a high-affinity antibody receptor expressed on macrophages and to a lesser extent on neutrophils. Like other antibody receptors, CD64 binds the Fc region of antibody molecules and so is called an Fc receptor (Fc γ RI). Its expression is enhanced by IFN- γ -induced activation. Human macrophages also carry two low-affinity antibody receptors, CD32 (Fc γ RII) and CD16 (Fc γ RIII). Cattle macrophages have a unique Fc receptor called Fc γ 2R, which can bind particles coated with a specific type of antibody called IgG2.

Macrophages also have receptors for complement components. They include CD35 (CR1), the major receptor for C3b, and the integrin CD11b/CD18, which is also a receptor for fragments of C3b. These receptors permit macrophages to bind organisms coated with C3b.

The integrins described in the previous chapter are responsible for binding macrophages to other cells, to

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FIGURE 4-3 The progressive activation of macrophages can involve three pathways. Thus macrophages can become classically activated M1 cells by exposure to microbial products and/or to Th1 cytokines such as interferon- γ (*IFN*- γ). Alternatively they may undergo “alternative activation” on exposure to Th2 cytokines and so become M2 cells.

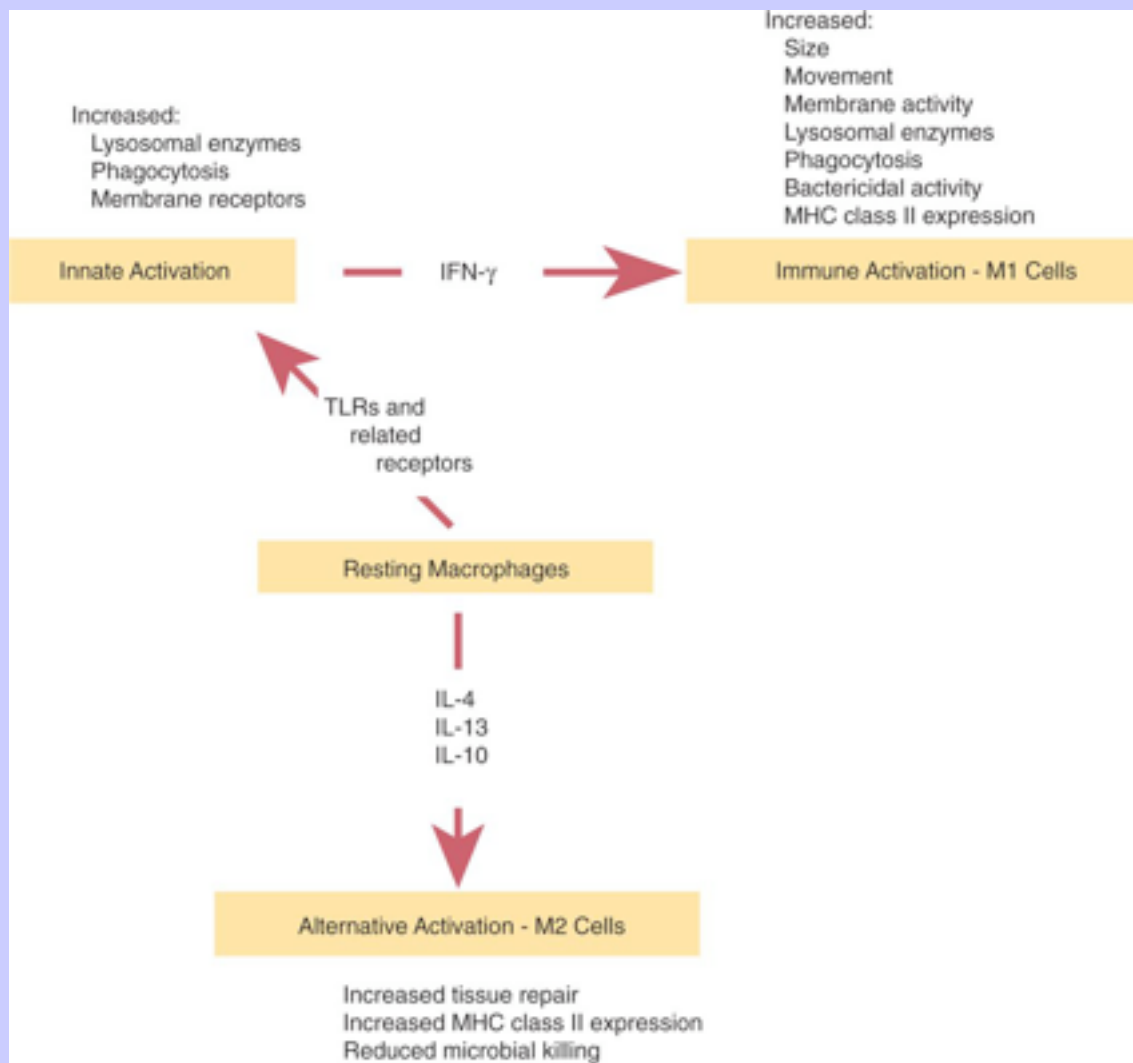
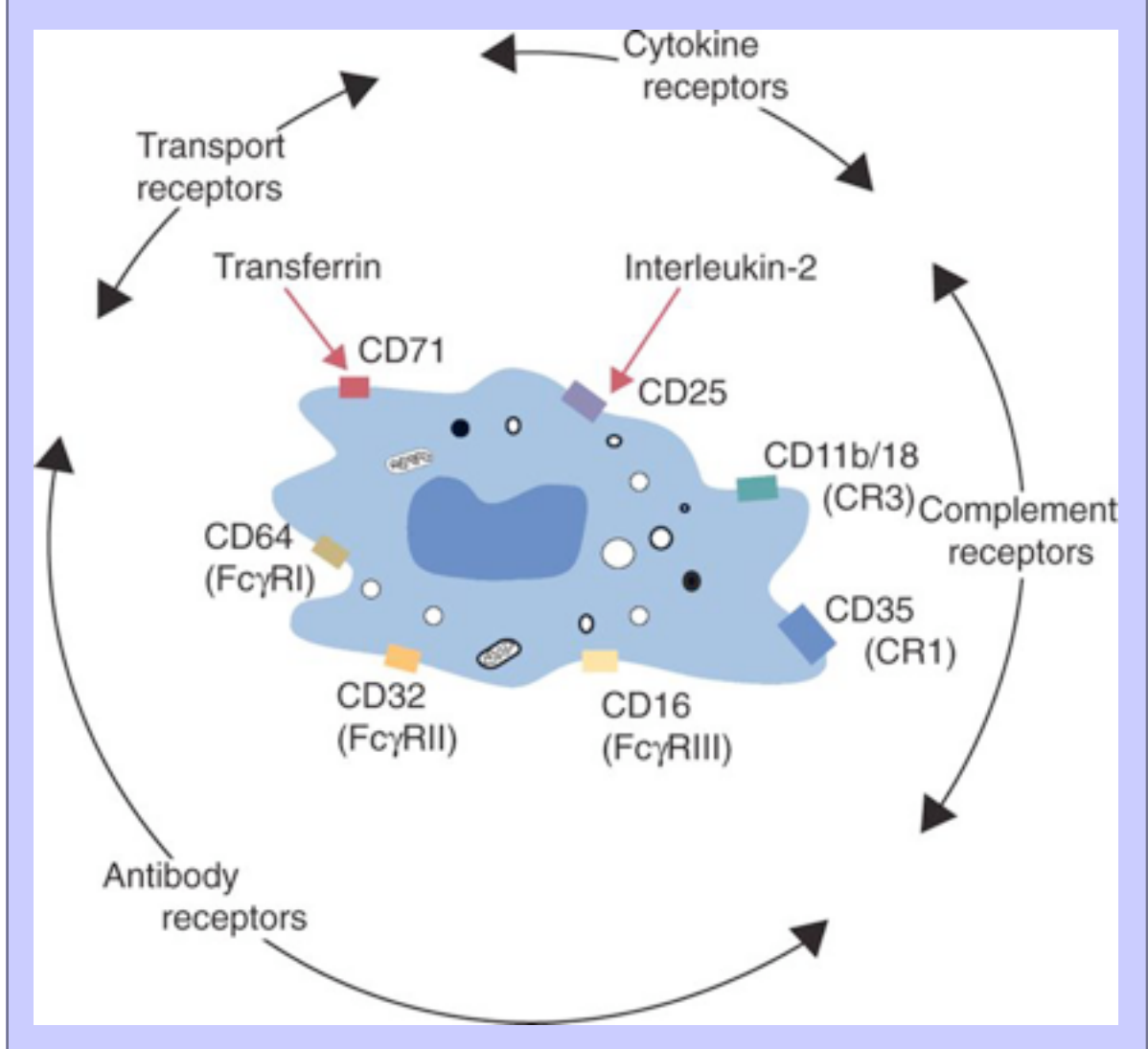


FIGURE 4-4 Some of the major surface receptors expressed on macrophages and their functions.



connective tissue molecules such as collagen and fibronectin, and to some complement components. Macrophages also have mannose-binding receptors (CD206) that can bind to mannose or fucose in the capsule or lipopolysaccharide of invading bacteria and so permit macrophages to bind and ingest non-opsonized bacteria.

Another important macrophage receptor is CD40. This glycoprotein is used to communicate with lymphocytes. Its ligand is called CD40 ligand (CD40L or CD154) and is found on T cells. Macrophages also receive activation signals via CD40.

4.3 THE FATE OF FOREIGN MATERIAL

Macrophages are located throughout the body and hence can capture invaders entering by many different routes. For example, if bacteria are injected intravenously, they are rapidly removed from the blood. Their precise fate depends on the species involved. In

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FIGURE 4-5 The different routes by which bacteria are cleared from the bloodstream in the dog and cat. Dogs mainly use Kupffer cells in the liver. Cats mainly employ pulmonary intravascular macrophages.

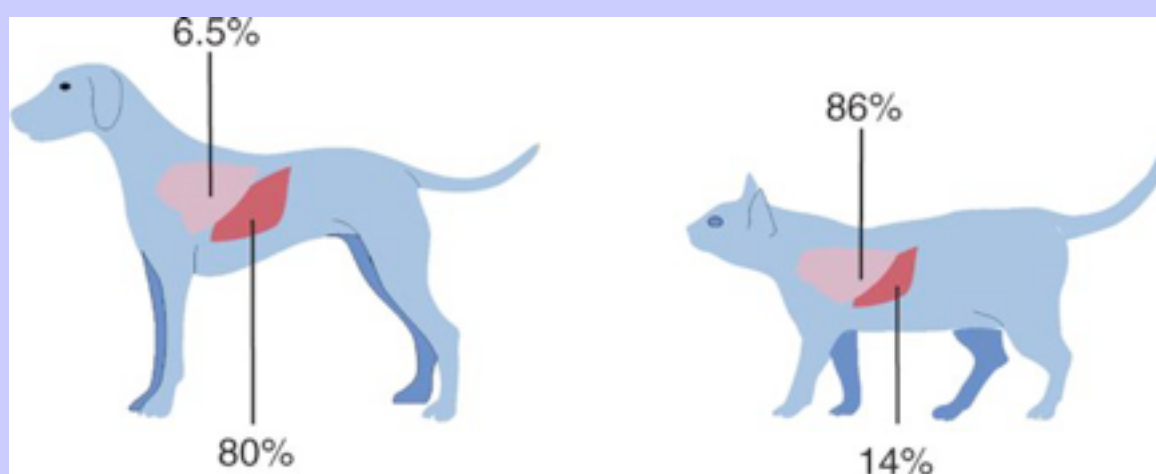


FIGURE 4-6 An intravascular macrophage (*M*) from the lung of a 7-day-old pig. The cell has numerous pseudopods, electron-dense siderosomes, phagosomes, and lipid droplets. It is closely attached to the thick portion of the air-blood tissue barrier that contains fibroblasts (*F*) and a pericyte (*P*) between basal laminae of the capillary endothelium (*E*) and the alveolar epithelium. At sites of close adherence, intercellular junctions with subplasmalemmal densities are seen (arrow). Bar = 2 μm ($\times 8000$). (From Winkler GC, Cheville NF: *Microvasc Res* 33:224-232, 1987.)

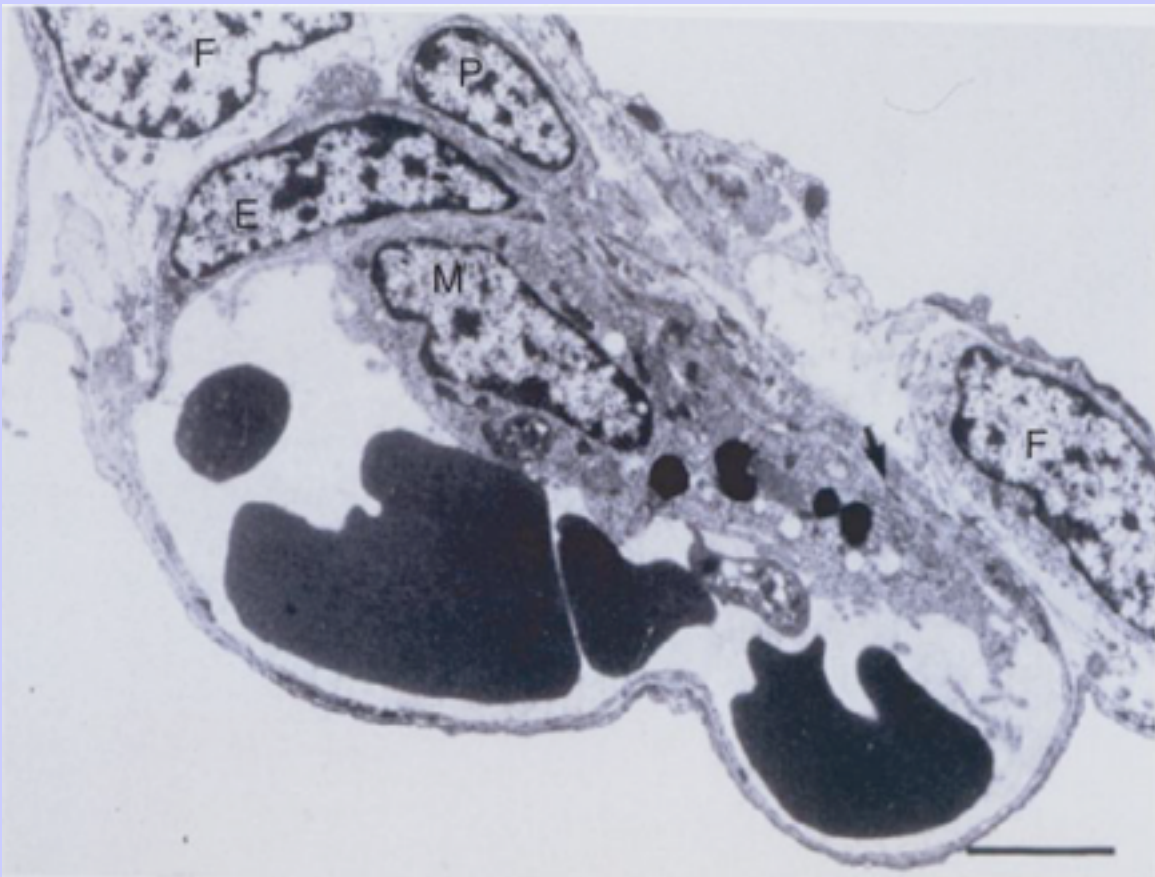
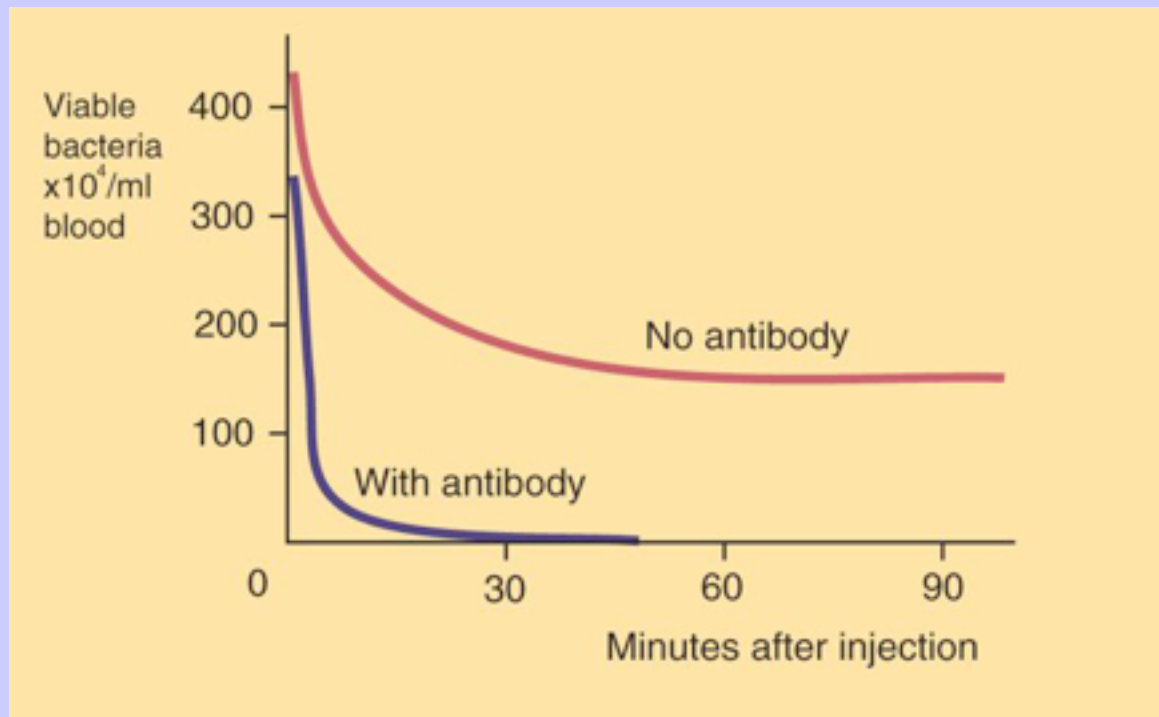


FIGURE 4-7 The clearance of bacteria from the blood (in this case *Escherichia coli* from piglets). In the absence of antibodies, bacteria are slowly and incompletely removed.



dogs, rodents, and humans, foreign particles are predominantly (80% to 90%) trapped and removed in the liver. Particles such as bacteria are removed by the macrophages (Kupffer cells) that line the sinusoids of the liver. The process occurs in two stages. Bacteria are first phagocytosed by neutrophils. These neutrophils are then ingested and destroyed by the Kupffer cells. These processes thus resemble acute inflammation, where neutrophils are primarily responsible for destruction of invaders, whereas macrophages are responsible for preventing damage caused by dying neutrophils ([Table 4-1](#)). In ruminants, pigs, horses, and cats, particles are mainly removed from the bloodstream by pulmonary intravascular macrophages ([Figure 4-5](#)). These macrophages line the endothelium of lung capillaries ([Figure 4-6](#)).

Table 4-1 Sites of Clearance of Particles from the Blood in Domestic Mammals

Species	Localization (%)	
	Lung	Liver/Spleen
Calf	93	6
Sheep	94	6
Dog	6.5	80
Cat	86	14
Rabbit	0.6	83
Guinea pig	1.5	82
Rat	0.5	97
Mouse	1.0	94
Selected data from Winkler GC: <i>Am J Anat</i> 181:223, 1988; and Chitko-McKown CG, Blecha F: <i>Ann Rech Vet</i> 23:201-214, 1992.		

In species in which hepatic clearance is important, large viruses or bacteria may be cleared completely by a single passage through the liver ([Figure 4-7](#)). The spleen is a more effective filter than the liver, but since it is much smaller, it traps much less material. There are also differences in the type of particle removed by the liver and spleen. Splenic macrophages have antibody receptors (CD64) so that particles opsonized with antibody are preferentially removed in the spleen. In contrast, phagocytic cells in the liver express CD35, a receptor for C3, the third component of complement, so that particles opsonized by C3 are preferentially removed in the liver. The clearance of particles from the blood is regulated by opsonins such as fibronectin or mannose-binding lectin. If an animal is injected intravenously with a very large dose of colloidal carbon, these opsonins will be temporarily depleted and other particles (such as bacteria) will not be removed from the bloodstream. In this situation the mononuclear-phagocytic system is said to be “blockaded.”

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Removal of organisms from the blood is greatly enhanced if they are opsonized by specific antibodies. If antibodies are absent or the bacteria possess an antiphagocytic polysaccharide capsule, the rate of clearance is decreased. Some molecules, such as bacterial endotoxins, estrogens, and simple lipids, stimulate macrophage activity and therefore increase the rate of bacterial clearance. Steroids and drugs that depress macrophage activity depress the clearance rate.

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4.3.1 Soluble Proteins Given Intravenously

Unless carefully treated, protein molecules in solution tend to aggregate spontaneously. If a protein solution is injected intravenously, neutrophils, monocytes, and macrophages rapidly remove these protein aggregates. The unaggregated protein remains in solution and is distributed evenly through the animal's blood. Small proteins (less than 60 kDa) also spread throughout the extravascular tissue fluids. Once distributed, the protein is catabolized, resulting in a slow but progressive decline in its concentration. Within a few days, however, the animal mounts an immune response. Antibodies combine with the foreign antigen. Phagocytic cells remove these antigen-antibody complexes from the blood, and the protein is rapidly eliminated ([Figure 4-8](#)).

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This triphasic clearance pattern of distribution, catabolism, and immune elimination may be modified under certain circumstances. For example, if the animal has not been previously exposed to an antigen, it takes between 5 and 10 days before immune elimination occurs. If, on the other hand, the animal has been primed by prior exposure to the antigen, a secondary immune response will occur in 2 to 3 days, and the stage of progressive catabolism will therefore be short. If antibodies are present at the time of antigen administration, immune elimination is immediate, and no catabolic phase is seen. If the injected material is not antigenic, or if an immune response does not occur, catabolism will continue until all the material is eliminated.

4.3.2

Fate of Material Administered by Other Routes

When foreign material is injected into a tissue, some damage and inflammation are bound to occur and alarmins are released. As a result, neutrophils and macrophages migrate toward the injection site and phagocytose the injected material. Some will, however, be captured by dendritic cells. The material taken up by macrophages and dendritic cells is processed and used to trigger acquired immunity. Antibodies and complement (see [Chapter 5](#)) interact with the antigenic material, generating chemotactic factors that attract still more phagocytic cells, thus hastening its final elimination. In the skin, a web of antigen-trapping dendritic cells called Langerhans cells may trap foreign molecules and present them directly to lymphocytes. For this reason intradermal injection of antigen may be most effective in stimulating an immune response.

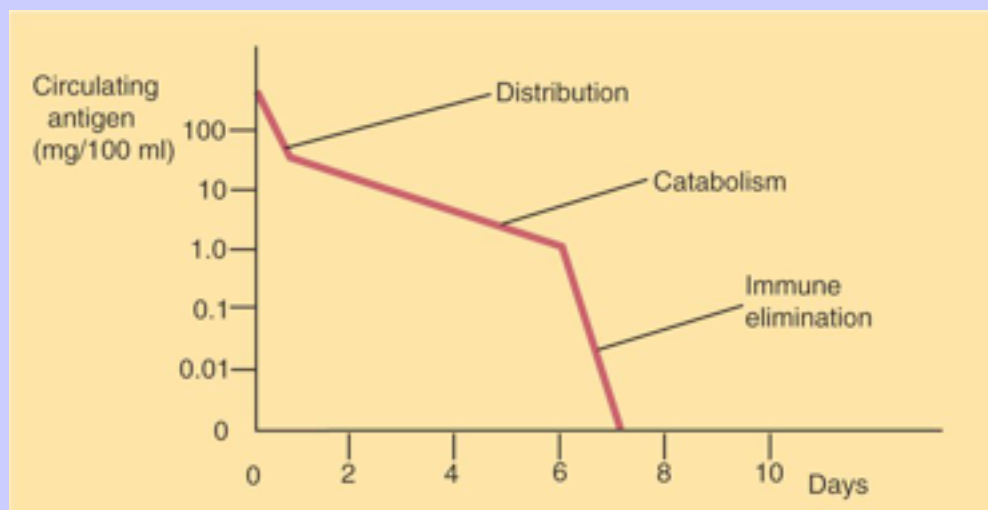
Soluble material injected into a tissue is redistributed by the flow of tissue fluid through the lymphatic system. It eventually reaches the bloodstream, so its final fate is similar to intravenously injected material. Any aggregated material present is phagocytosed by neutrophils or tissue macrophages or by the macrophages and dendritic cells of lymph nodes through which the tissue fluid flows.

4.3.2.1

Digestive Tract

Digestive enzymes normally break molecules passing through the intestine into small fragments. However,

FIGURE 4-8 The clearance of a soluble antigen from the bloodstream. Note the three phases of this clearance.



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some molecules may remain intact and pass through the intestinal epithelium. Bacterial polysaccharides and molecules that associate with lipids are especially effective in this respect, since they are absorbed in chylomicrons. Particles that enter the blood from the intestine are removed by macrophages in the liver, whereas those particles entering the intestinal lymphatics are trapped in the mesenteric lymph nodes.

4.3.2.2

Respiratory Tract

The fate of inhaled particles depends on their size. Large particles (greater than 5 μm diameter) stick to the mucous layer that covers the respiratory epithelium from the trachea to the terminal bronchioles (see [Chapter 19, Figure 19-3](#)). These particles are then removed by the flow of mucus toward the pharynx or by coughing. Very small particles that reach the lung alveoli are ingested by alveolar macrophages, which carry them back to the bronchoalveolar junction; from there they are also removed by the flow of mucus. Nevertheless, some material may be absorbed from the alveoli. Small particles absorbed in this way are cleared to the draining lymph nodes, whereas soluble molecules enter the bloodstream and are distributed throughout the body. When large quantities of particles are inhaled, as occurs in workers exposed to industrial dusts or in cigarette smokers, the alveolar macrophage system may be “blockaded” and the lungs made more susceptible to microbial invasion.

4.4

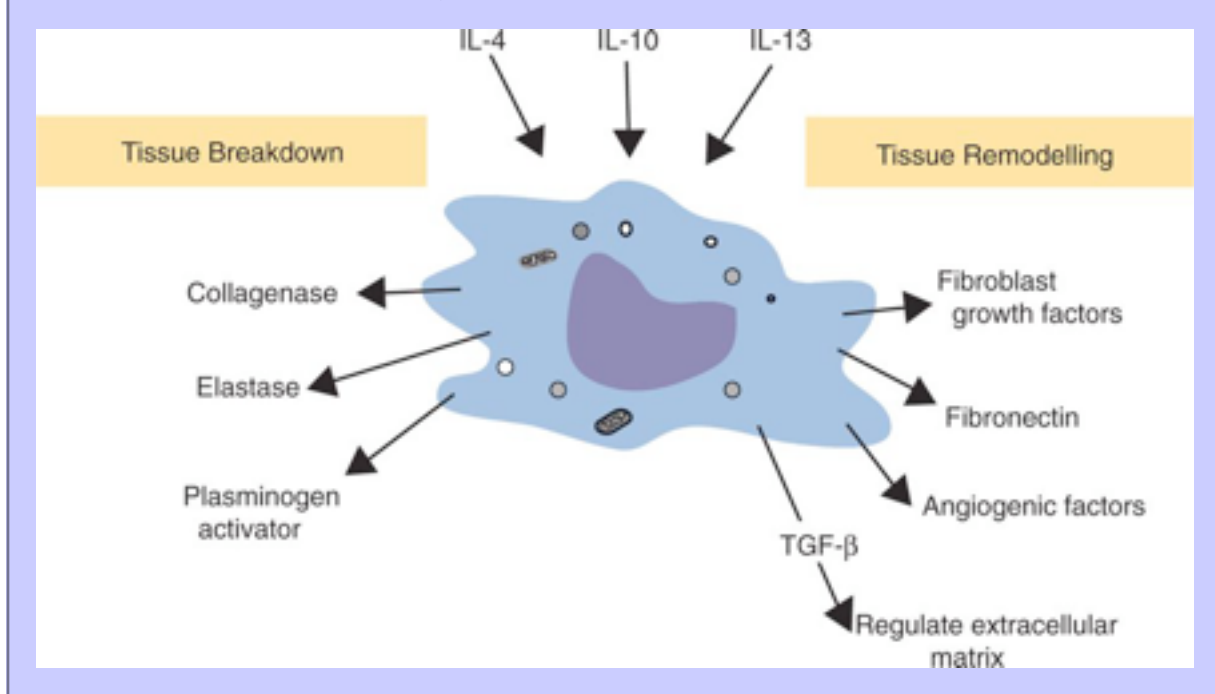
RECOVERY FROM INFLAMMATION

Once invading organisms have been destroyed, the tissue response must switch from a killing process to a repair process. Thus as inflammation progresses, macrophages change their properties ([Figure 4-9](#)). They are first activated in the classical manner by $\text{TNF-}\alpha$ in order to kill ingested bacteria. However these M1 macrophages eventually convert to M2 cells and develop antiinflammatory properties. Thus the same cell can act in a proinflammatory manner at the beginning of an infection but switch to antiinflammatory activities once the infection is overcome.

Once they switch, M2 cells secrete SLP1, a serine protease inhibitor. This molecule inhibits the release of elastase and oxidants by $\text{TNF-}\alpha$ -stimulated neutrophils and inhibits the activity of the elastase. SLP1 also protects the antiinflammatory cytokine transforming growth factor- β (TGF- β) from breakdown, and TGF- β inhibits the release of $\text{TNF-}\alpha$. Neutrophils also change during inflammation. Thus they secrete fragments of the $\text{TNF-}\alpha$ receptor that can bind and neutralize $\text{TNF-}\alpha$. $\text{TNF-}\alpha$ stimulates macrophages to secrete IL-12, which in turn induces lymphocytes to secrete IFN- γ . The IFN- γ acts as a macrophage activator early in the inflammatory process, but later it becomes suppressive. Neutrophil-derived lipoxins suppress leukotriene synthesis.

Even in normal healthy animals, many cells die every day and must be promptly removed. Much of this task is the function of macrophages. A good example of this is the daily removal of enormous numbers of aged neutrophils. Macrophages, it appears, methodically “palpate” any neutrophils that they encounter. If the neutrophil is healthy, it quickly detaches from the macrophage. If, however, the neutrophil is dead or dying, the macrophage remains in contact and eats the neutrophil. This interaction operates through the adhesion protein CD31 ([Figure 4-10](#)). Thus CD31 on a neutrophil binds to CD31 on a macrophage. If the neutrophil is healthy, it sends a signal to

FIGURE 4-9 The role of M2 macrophages in tissue breakdown and tissue repair in wound healing.



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FIGURE 4-10 The removal of apoptotic neutrophils. The reaction is initiated by interactions between CD31 on neutrophils and macrophages. If the neutrophil fails to reply when interrogated by a macrophage, it will be ingested and destroyed.

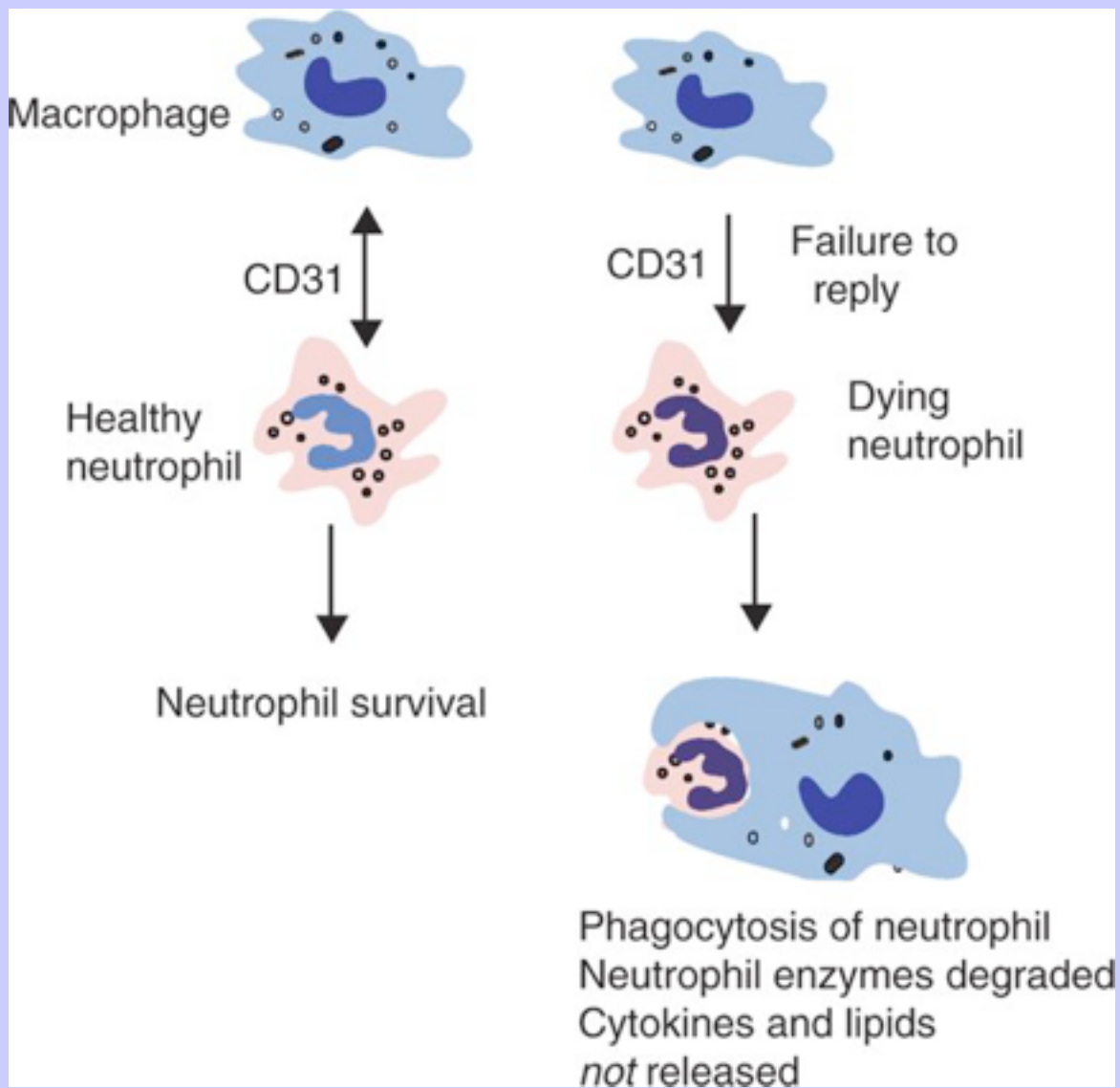
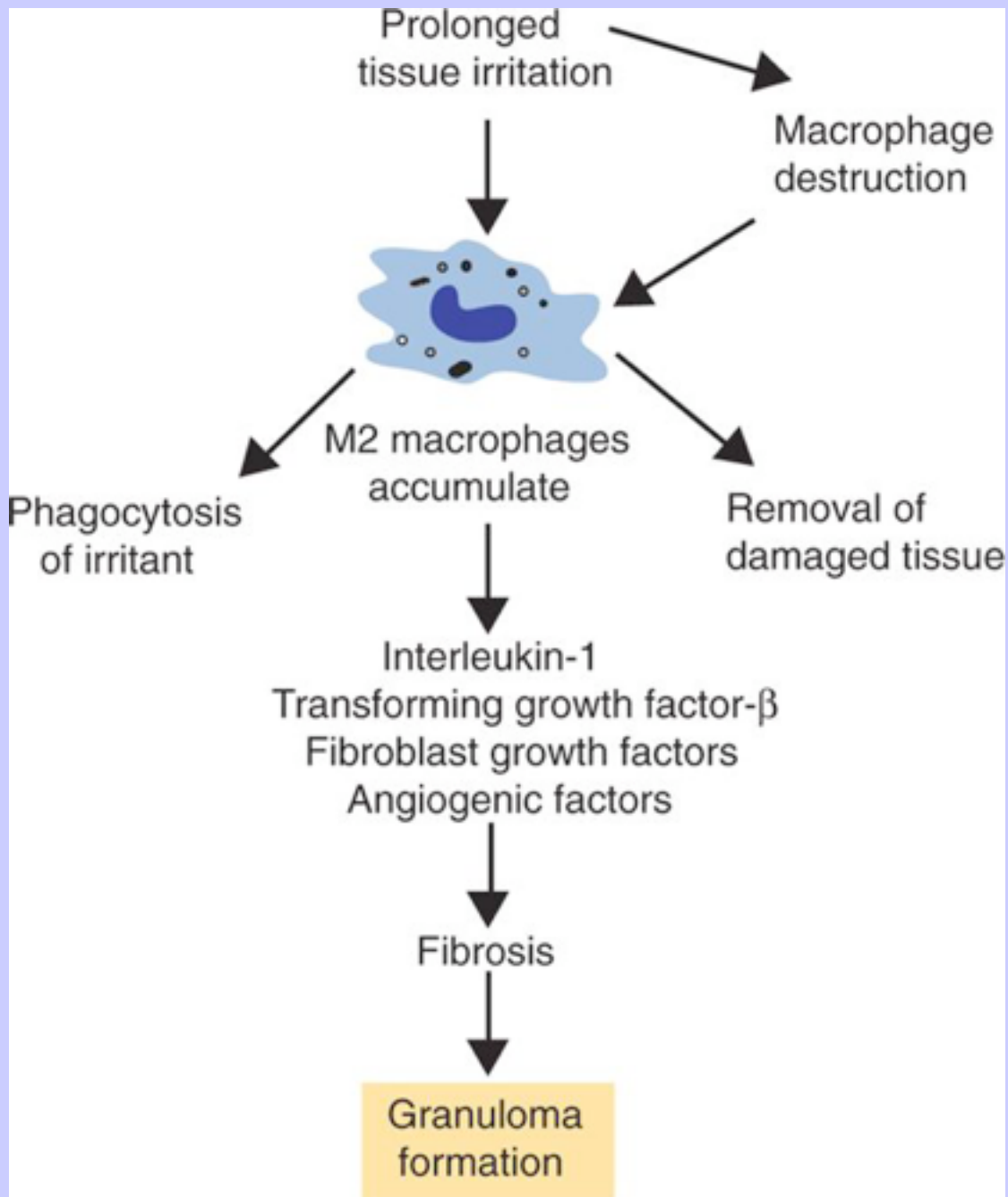


FIGURE 4-11 The pathogenesis of chronic inflammation. Macrophages undergoing prolonged stimulation may switch from an M1 to an M2 phenotype. M2 cells secrete cytokine mixtures that not only promote wound healing but also promote the “walling off” of persistent irritants by fibroblasts and extracellular matrix.



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the macrophage, causing it to disengage. On the other hand, if the neutrophil fails to signal, it will be eaten. It is interesting to note that this failure in CD31 signaling occurs long before a neutrophil becomes so degraded that its contents can leak and cause damage. The macrophages that consume these neutrophils do not release cytokines or vasoactive lipids. Ingestion of apoptotic neutrophils does, however, cause the macrophages to secrete more TGF- β , which in turn promotes tissue repair. Phagocytosis is thus an efficient way of removing apoptotic neutrophils without causing additional tissue damage or inflammation.

By secreting IL-1 β , macrophages attract and activate fibroblasts. The fibroblasts enter the damaged area and secrete collagen. Once sufficient collagen has been deposited, its synthesis stops. This collagen is then remodeled over several weeks or months as the area returns to normal. The reduced oxygen tension in dead tissues stimulates macrophages to secrete cytokines that promote the growth of new blood vessels. Once the oxygen tension is restored to normal, new blood vessel formation ceases.

The final result of this healing process depends on the effectiveness of the inflammatory response. If the cause is rapidly and completely removed, healing will follow uneventfully.

If tissue health is not restored, either because the invaders are not eliminated or because tissue repair is inadequate, inflammation may persist and become a damaging, chronic condition. Examples of persistent invaders include bacteria such as *M. tuberculosis*, fungi such as *Cryptococcus*, parasites such as liver fluke, or inorganic material such as asbestos crystals. Macrophages, fibroblasts, and lymphocytes may accumulate in large numbers around the persistent material over months or years. Because they resemble epithelium in histological sections, these accumulated macrophages are called epithelioid cells. Epithelioid cells may fuse and form multinucleated giant cells if they attempt to enclose particles too large to be ingested by a single macrophage. Epithelioid cells and giant cells are a prominent feature of tubercles, the persistent inflammatory lesions that develop in individuals suffering from tuberculosis (see [Chapter 28](#)).

In all these cases, the persistence of foreign material results in the continual arrival of new M2 macrophages, which continue to attract fibroblasts and stimulate the deposition of collagen. The chronic inflammatory lesion that develops around the foreign material is called a granuloma ([Figure 4-11](#)). Granulomas consist of granulation tissue—an accumulation of macrophages, lymphocytes, fibroblasts, loose connective tissue, and new blood vessels. The term *granulation* tissue is derived from the granular appearance of this tissue when cut. The “granules” are in fact new blood vessels.

If the persistent irritant is a nonantigenic “foreign body” (for example, silica, talc, or mineral oil), few neutrophils or lymphocytes will be attracted to the lesion. Epithelioid and giant cells, however, attempt to destroy the offending material. If the material is toxic for macrophages (as is asbestos), leaking enzymes may lead to progressive tissue damage, local fibrosis, and scarring.

If the irritant is antigenic, the granuloma may contain many lymphocytes as well as macrophages, fibroblasts, and probably some neutrophils, eosinophils, and basophils ([Figure 4-12](#)). The chronically activated M2 cells within these granulomas secrete IL-1, which stimulates collagen deposition by fibroblasts and eventually “walls off” the lesion from the rest of the body. Granulomas are produced in response to bacteria such as the mycobacteria and *Brucella abortus* and parasites such as liver fluke and schistosomes. Chronic granulomas, whether due to immunological or foreign body reactions, are important since they may enlarge and destroy normal tissues. In liver fluke infestations, for example, death may result from the gradual replacement of normal liver cells by fibrous tissue formed as a result of the persistence of the parasites.

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4.5 SICKNESS BEHAVIOR

When an animal is invaded by microorganisms, a generalized response occurs—a response that we call sickness. The subjective feelings of sickness—malaise, lassitude, fatigue, loss of appetite, and muscle and joint pains—along with a fever, are components of innate immunity. They reflect a change in the body's priorities as it seeks to fight off invaders. Microbial molecules acting on the TLRs of phagocytic cells stimulate the production of IL-1 β , IL-6, and TNF- α , which affect the brain ([Figure 4-13](#)). These cytokines signal to the brain by two routes. One route is direct through neurons that serve damaged tissue. IL-1 receptors are found on sensory neurons on the vagus nerve, and vagal sensory stimulation can thus trigger sickness responses in the brain. (IL-1 β can make the vagus nerve excessively sensitive and so trigger nausea.) The second route involves circulating cytokines that either diffuse into the brain or are produced within the

FIGURE 4-12 A granulomatous inflammatory reaction around a degenerating tapeworm cyst in a bovine heart. The mass of cells around the central organism is a mixture of macrophages and fibroblasts serving to wall it off from the rest of the body ($\times 250$). (Courtesy Dr. John Edwards.)

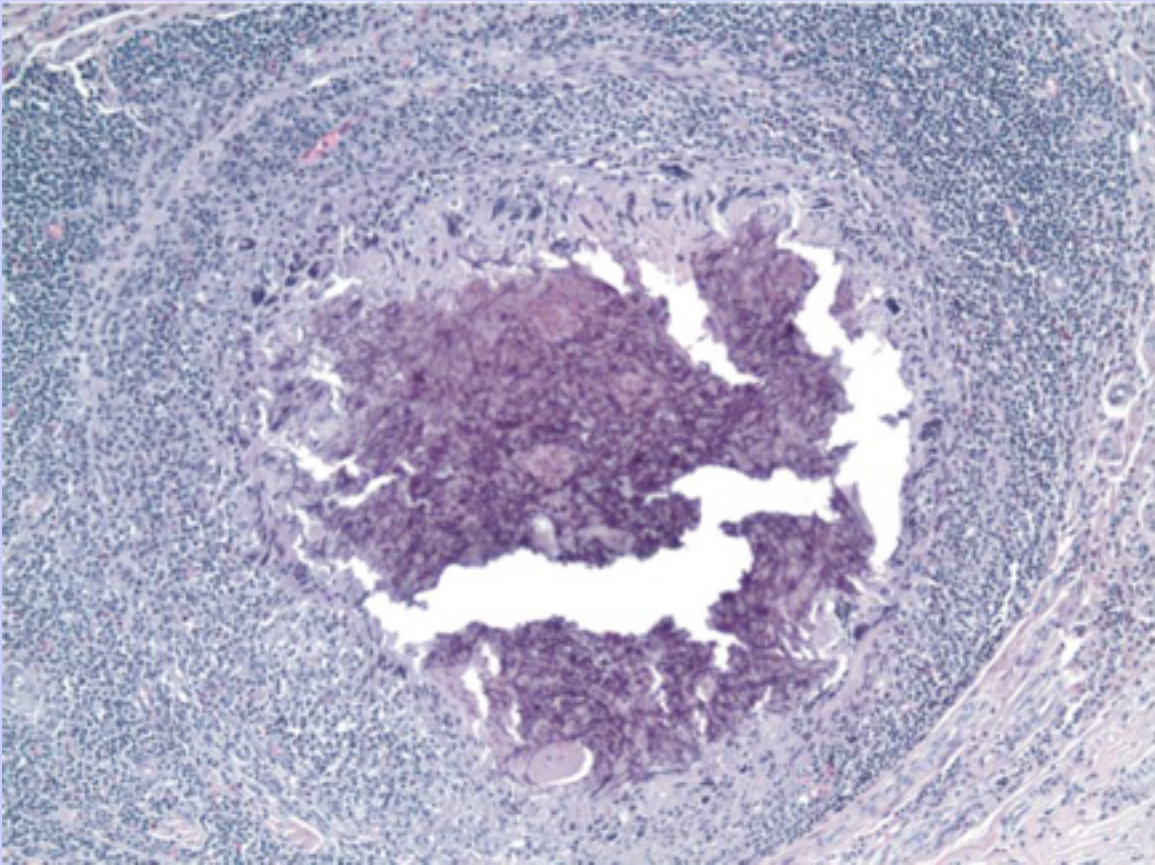
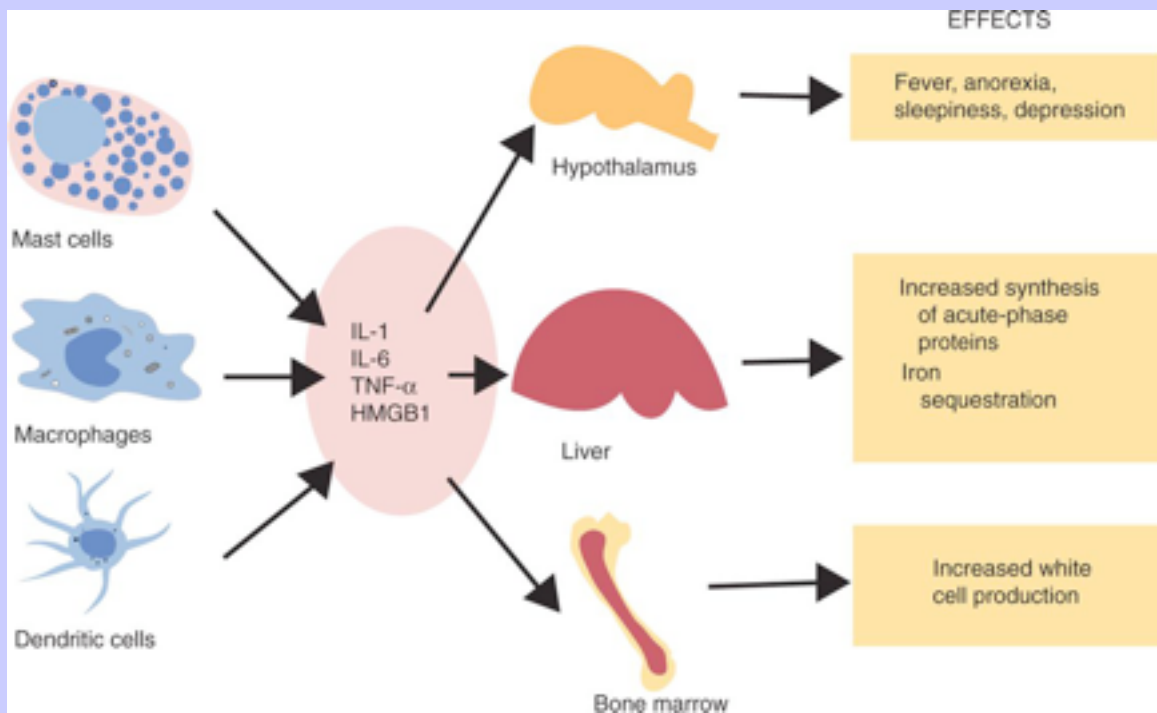


FIGURE 4-13 Sickness behavior is part of the response of the body to inflammatory stimuli. Multiple systemic effects are due to the four major cytokines secreted by sentinel cells, mast cells, macrophages, and dendritic cells. The major sickness-inducing cytokines are interleukin-1 (*IL-1*), IL-6, tumor necrosis factor- α (*TNF- α*), and high mobility group box protein-1 (*HMGB1*).



brain. These cytokines act on the brain to modify behavior.

One of the most obvious features of the brain's response to infection is the development of a fever. IL-1, IL-6, and TNF- α all act on the brain to induce sleep, suppress appetite, and raise body temperature (except in mice, whose temperature drops). These cytokines induce prostaglandin production, which causes the body's thermostatic set-point to rise. In response, animals conserve heat by vasoconstriction and increase heat production by shivering, thus causing the body temperature to rise until it reaches the new set-point. This fever enhances some components of the immune responses. For example, elevated body temperatures cause dendritic cells to mature, enhance the circulation of lymphocytes, and promote the secretion of IL-2. Fever range temperatures greatly enhance the survival of T cells by inhibiting their apoptosis. The cytokines released during inflammation, especially IL-1, are also responsible for the reduction in social behavior seen in sickness; they promote the release of sleep-inducing molecules in the brain. Increased lethargy is commonly associated with a fever and may, by reducing the energy demands of an animal, increase the efficiency of defense and repair mechanisms. IL-1 also suppresses the hunger centers of the brain and so induces the loss of appetite associated with infections. The benefits of this are unclear, but it may permit the animal to be more selective about its food. If the anorexia persists, it can have an adverse effect on growth.

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High mobility group box protein-1 (HMGB1) is a potent sickness-inducing cytokine. Although IL-1, IL-6, and TNF- α have long been known to be mediators of septic shock and sickness behavior, it is now clear that these three molecules plus IFN- γ induce HMGB1 release from macrophages several hours after initiation of sickness. It enters secretory lysosomes and is released slowly from the cells. HMGB1 has been implicated in food aversion and weight loss by its actions on the hypothalamic-pituitary axis. It mediates endotoxin lethality, arthritis, and macrophage activation. It is likely that the inflammation induced by necrotic cells is caused by the release of HMGB1 from disrupted nuclei. HMGB1 is an excellent example of an alarmin.

4.5.1

Metabolic Changes

In addition to their effects on the nervous and immune systems, IL-1, IL-6, and TNF- α act on skeletal muscle to enhance protein catabolism and thus mobilize a pool of available amino acids. Although this eventually results in muscle wastage, the newly available amino acids are available for increased antibody synthesis. Other systemic responses include the development of a neutrophilia (elevated blood neutrophils) as a result of enhanced stem cell activity, weight loss due to muscle wasting and loss of adipose tissue, and the production of many new proteins (acute-phase proteins) that help fight infection.

Animals exposed to chronic, sublethal doses of TNF- α lose weight and become anemic and protein depleted. The weight loss occurs because TNF- α inhibits the synthesis of enzymes necessary for the uptake of lipids by preadipocytes and causes mature adipocytes to lose stored lipids. TNF- α is thus responsible for the weight loss seen in animals with cancer or chronic parasitic and bacterial diseases.

4.5.2

Acute-Phase Proteins

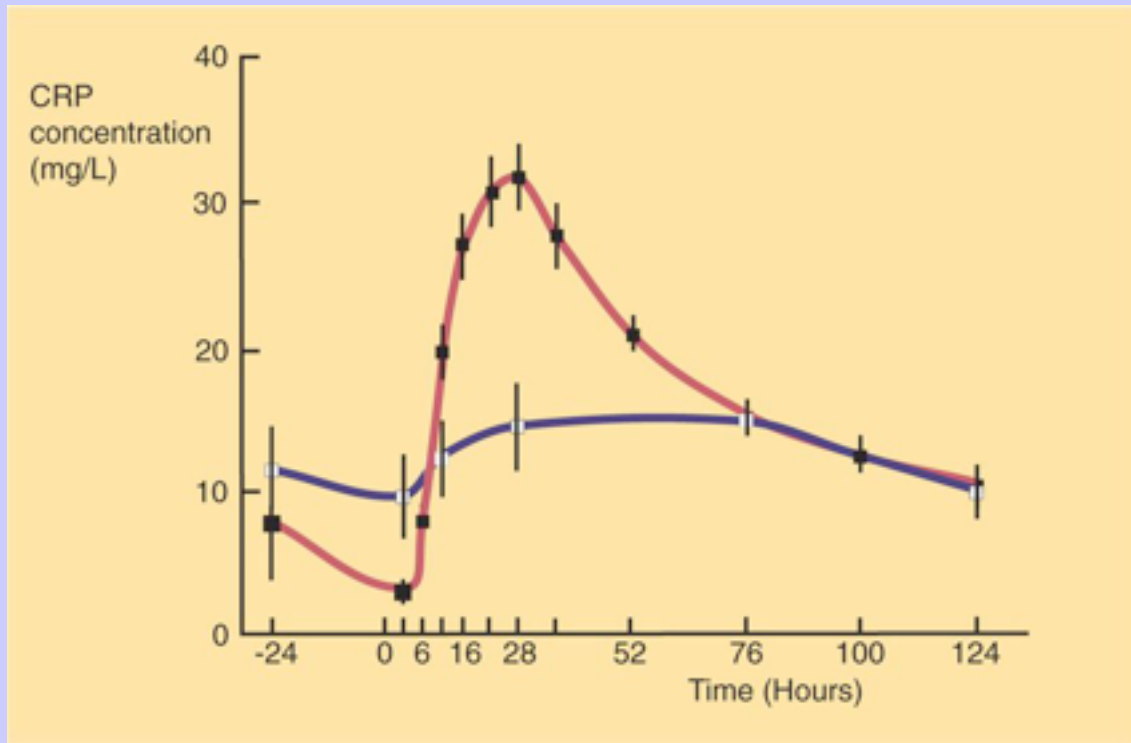
Under the influence of IL-1 β , TNF- α , and especially IL-6, liver cells greatly increase their protein synthesis and secretion. This begins within a few hours of injury and subsides within 24 to 48 hours ([Figure 4-14](#)). Because this is associated with acute infections and inflammation, the newly produced proteins are called acute-phase proteins. Many of the acute-phase proteins are important components of the innate immune system. They include complement components, clotting molecules, protease inhibitors, and metal-binding proteins. Different mammals produce different acute-phase proteins ([Figure 4-15](#)).

C-reactive protein (CRP) is the major acute-phase protein in primates, rabbits, hamsters, and dogs and is important in pigs. CRP is a pentraxin and so has a pentameric structure with two faces. One face binds to phosphocholine, a common side chain found in all cell membranes and many bacteria and protozoa. The other face is responsible for binding to neutrophils through the antibody receptors Fc γ RI and Fc γ RIIa and to the complement component C1q. CRP can thus promote the phagocytosis and removal of damaged, dying, or dead cells as well as microorganisms. CRP can bind to bacterial polysaccharides and glycolipids and to healthy and damaged cells, where it activates C1q and the classical complement pathway. (Its name derives from its ability to bind and precipitate the C-polysaccharide of *Streptococcus pneumoniae*.) CRP also has an antiinflammatory role since it inhibits neutrophil superoxide production and degranulation and blocks platelet aggregation. CRP may therefore promote healing by reducing damage and enhancing the repair of damaged tissue. The functions of CRP differ between species. For example, in cattle, the level of CRP rises twofold to fivefold in lactating cows.

Serum amyloid A (SAA) is the major acute-phase protein in cattle, cats, and horses and is also important in humans and dogs. Thus equine SAA concentrations rise several hundred-fold during noninfectious arthritis, while canine SAA concentrations increase up to twentyfold in bacterial disease. Since SAA protein

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FIGURE 4-14 The rise in C-reactive protein levels in six dogs following anesthesia and surgery (red line) and in six dogs undergoing anesthesia alone (blue line). (From Burton SA, Honor DJ, Mackenzie AL, et al: *Am J Vet Res* 55:615, 1994.)



is immunosuppressive, it probably regulates immune responses. SAA is a chemoattractant for neutrophils, monocytes, and T cells. SAA increases significantly in mastitic milk.

Serum amyloid P (SAP) is the major acute-phase protein in rodents. It is a pentraxin related to CRP. Like CRP, one face of the molecule can bind nuclear constituents such as DNA, chromatin, and histones as well as cell membrane phospholipids. The other face can also bind and activate C1q and thus activate the complement system.

Haptoglobin is a major acute-phase protein in ruminants, horses, and cats. It can rise from virtually undetectable levels in normal calves to as high as 1 mg/ml in calves with acute respiratory disease. Haptoglobin binds iron molecules and makes them unavailable to invading bacteria, thus inhibiting bacterial proliferation and invasion. Haptoglobin also binds free hemoglobin, thus preventing its oxidation of lipids and proteins. It is possible to identify animals with severe infections or inflammatory conditions by measuring serum haptoglobin levels. This may be of benefit in antemortem meat inspections by identifying those animals that are not fit to eat. Other iron-binding acute-phase proteins include transferrin (important in birds) and hemopexin.

Hepcidin is another iron-binding protein produced by hepatocytes under the influence of IL-6. Hepcidin suppresses intestinal iron absorption and macrophage iron release. As a result of the increase in hepcidin and haptoglobin, iron availability for red blood cell production drops and chronically infected animals become anemic—the anemia of infection.

Major acute-phase protein (MAP) is the major acute-phase protein in pigs and a substrate for the proteolytic enzyme kallikrein and so releases inflammatory peptides called kinins.

Other acute-phase proteins include lipopolysaccharide-binding protein (cattle); CD14 (humans and mice); collectins such as mannose-binding lectin and conglutinin (many species); ceruloplasmin and fibrinogen (sheep); and ceruloplasmin (pigs). Some serum protease inhibitors such as α_1 -antitrypsin, α_1 -antichymotrypsin, and α_2 -macroglobulin are acute-phase proteins in many mammalian species. All of these may inhibit neutrophil proteases in sites of acute inflammation.

Some protein levels fall during acute inflammation. These are called “negative” acute-phase proteins. In the pig, for example, these include albumin, fetuin, transferrin, transthyretin, and apolipoprotein A-1.

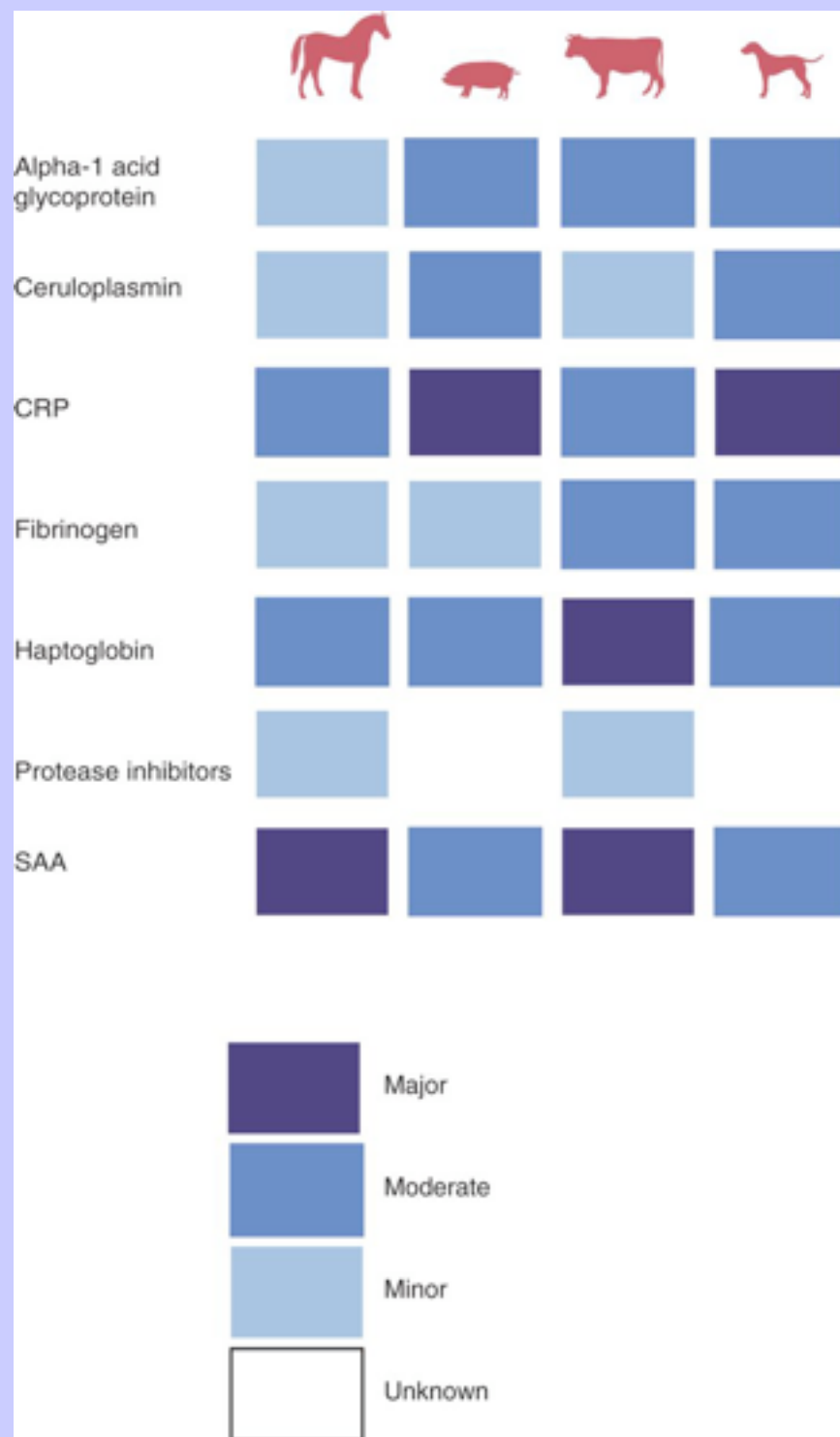
The two cytokines IL-1 and IL-6 have quite different effects on the liver and, as a result, are used to classify the acute phase proteins into two types. Type 1 acute phase proteins are those that require both IL-6 and IL-1 for maximum synthesis. Examples of type 1 acute phase proteins include CRP, SAA, and alpha-1 acid glycoprotein. Type 2 acute phase proteins, in contrast, require only IL-6 for maximum production. Examples of type 2 acute phase proteins include fibrinogen, haptoglobin, and alpha-2 macroglobulin.

4.6 SYSTEMIC INFLAMMATORY RESPONSE SYNDROME

In severe infections or after massive tissue damage, very large amounts of cytokines and oxidants may be produced, escape into the bloodstream, and cause

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FIGURE 4-15 Species differences in the major acute-phase proteins produced by the domestic mammals.



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a lethal form of shock known as systemic inflammatory response syndrome or, more simply, sepsis. Many different infectious diseases are characterized by the activation of large numbers of immune cells and the consequent production of large amounts of many different cytokines and inflammatory mediators within a short period of time. Since many cytokines are relatively toxic, this “cytokine storm” may cause severe toxicity, tissue damage, and even death. The most important of these include TNF- α , IFN- γ , IL-8, and IL-6. These cytokines may trigger the activation of additional T cells and the release of additional cytokines and therefore trigger a cytokine storm.

The most obvious of these cytokine storms is that resulting from tissue trauma, infections, or burns that give rise to septic shock. However, many important infections such as influenza, dengue, Gram-negative bacterial infections, filoviruses, and malaria may also trigger excessive cytokine release and death. Other diseases involving cytokine toxicity include graft-versus-host disease. Different triggers probably induce production of different cytokine mixtures at different sites so that the pathology of these diseases may be variable. Among the most important toxic effects is activation of endothelial cells leading to increased vascular permeability and intravascular coagulation.

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4.6.1

Bacterial Septic Shock

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Septic shock is the name given to the systemic inflammatory response syndrome caused by severe infections and associated with trauma, ischemia, and tissue injury. It accounts for about 9% of human deaths in the United States and is a correspondingly important cause of animal deaths. Animals or humans with mild infections develop the characteristic signs of sickness such as fevers, rigors, myalgia, depression, headache, and nausea as a result of cytokine release. Severe infections, however, may result in vastly excessive cytokine production that leads to severe acidosis, fever, lactate release in tissues, an uncontrollable drop in blood pressure, elevation of plasma catecholamines, and eventually to renal, hepatic, and lung injury and death. The procoagulant-anticoagulant balance is upset so that endothelial procoagulant activity is enhanced while many anticoagulant pathways are inhibited, leading to intravascular coagulation and capillary thrombosis ([Figure 4-16](#)).

All these effects are mediated by excessive triggering of TLRs leading to a massive and uncontrolled release of HMGB1 and other cytokines. TLRs 4 and 2 and HMGB1 receptors trigger a “cytokine storm,” from endotoxin-stimulated macrophages. Other cytokines involved include TNF- α and IL-1 β , with IFN- γ , IL-6, and CXCL8 (IL-8) in a supporting role. These cytokines in turn stimulate expression of NOS2 leading to an increase in serum nitric oxide and of cyclooxygenase-2, which in turn leads to prostaglandin and leuko-triene synthesis. The cytokines damage vascular endothelial cells, activating them so that procoagulant activity is enhanced, resulting in blood clotting. The nitric oxide causes vasodilation and a drop in blood pressure. The prostaglandins and leukotrienes cause increases in vascular permeability. The widespread damage to vascular endothelium eventually causes organ failure.

Multiple organ dysfunction syndrome is the end stage of severe septic shock. It is characterized by hypotension, insufficient tissue perfusion, uncontrollable bleeding, and organ failure caused by hypoxia, tissue acidosis, tissue necrosis, and severe local metabolic disturbances. The severe bleeding is due to disseminated intravascular coagulation.

The sensitivity of mammals to septic shock varies greatly. Species with pulmonary intravascular macrophages (cat, horse, sheep, and pig) tend to be more susceptible than dogs and rodents, which lack pulmonary intravascular macrophages and are thus relatively insusceptible to lung injury. It is of interest to note that in foals with sepsis, TLR4 gene expression is greatly increased and a poorer prognosis is associated with increased expression of IL-10.

Bacterial Toxic Shock

Some strains of *Staphylococcus aureus* produce enterotoxins that bind and stimulate T cell antigen receptors ([Figure 4-17](#)). These toxins may thus stimulate up to 20% of an animal's T cells, causing them to secrete enormous quantities of IL-2 and IFN- γ . These in turn stimulate production of TNF- α and IL-1 β . This leads to the development of a fever, hypotension, collapse, skin lesions, and damage to the liver, kidney, and intestines with multiple organ dysfunctions called toxic shock syndrome. A similar syndrome has also been observed in some streptococcal infections. In these cases, streptococcal M-protein binds to fibrinogen. The M-protein-fibrinogen complexes bind to endothelial cell integrins and trigger a respiratory burst. This causes an increase in vascular permeability and hypercoagulability leading to toxic shock characterized by hypotension and disseminated intravascular coagulation.

FIGURE 4-16 The pathogenesis of the systemic inflammatory response syndrome.

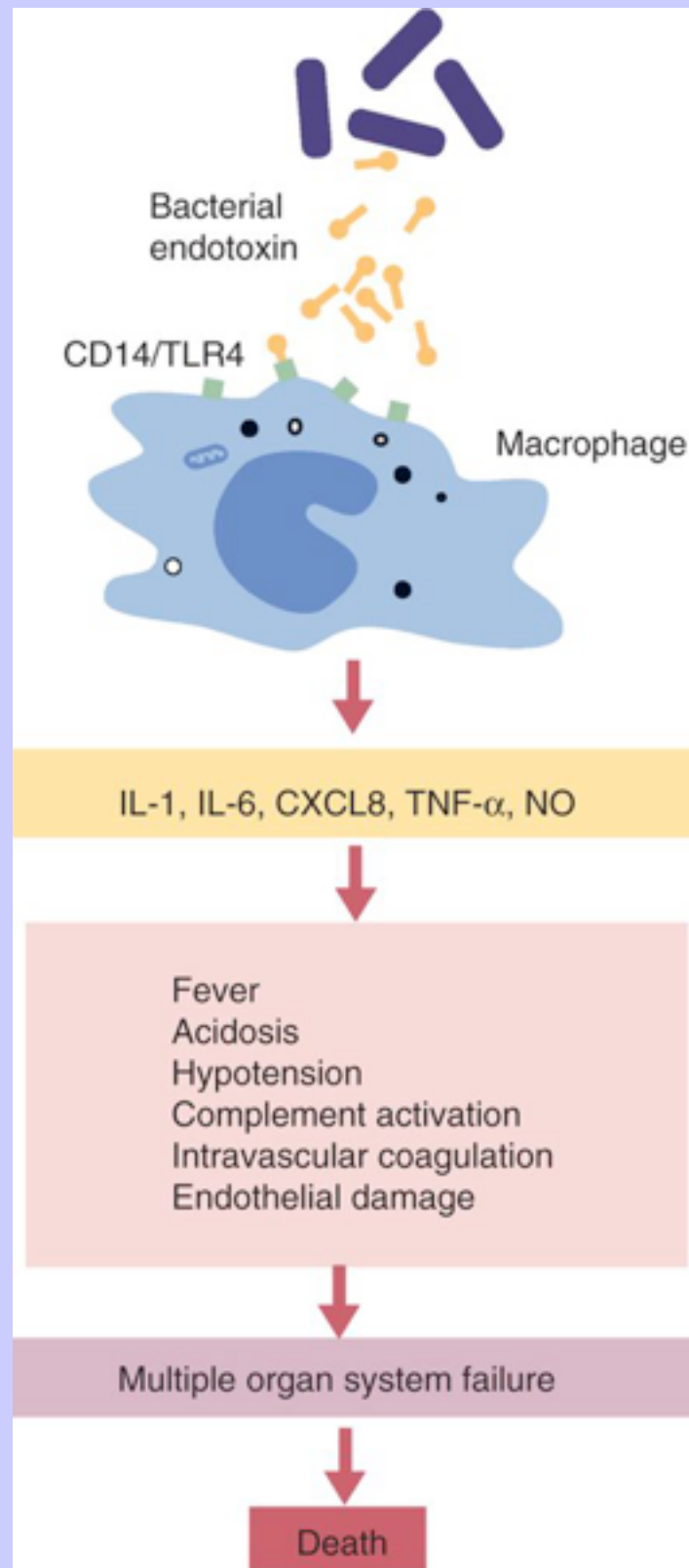


FIGURE 4-17 The pathogenesis of staphylococcal toxic shock syndrome.

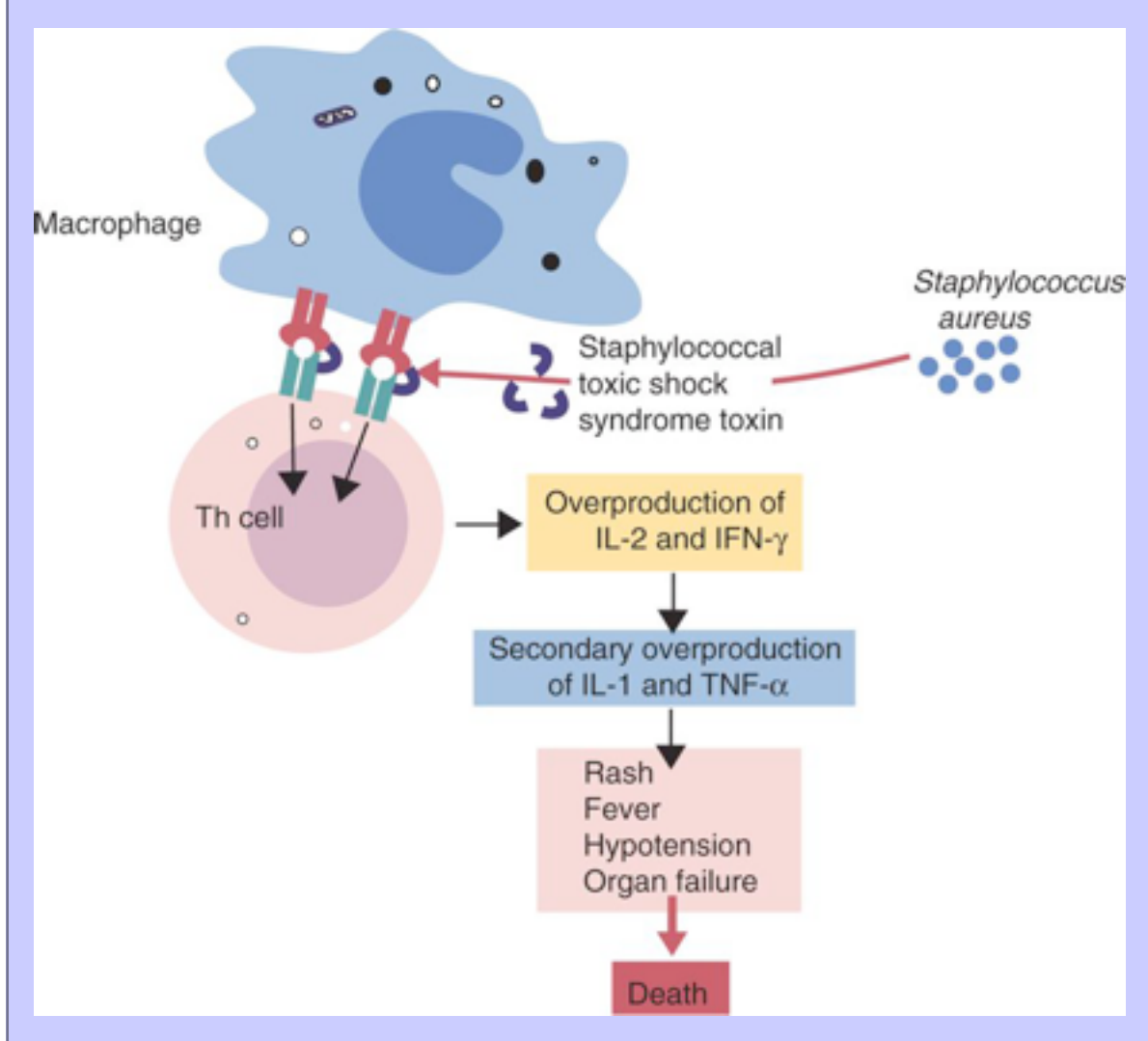
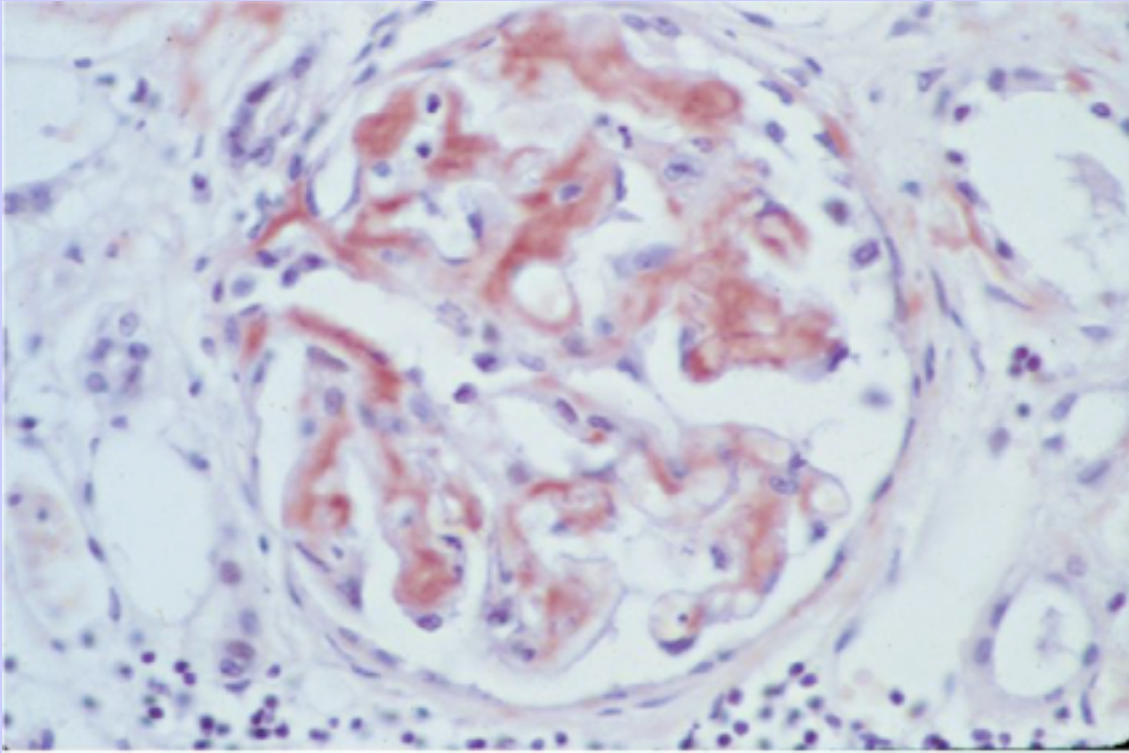


FIGURE 4-18 Secondary amyloid deposited in a glomerulus. The red dye (Congo red) specifically binds to amyloid fibrils ($\times 400$).



4.6.3 Graft-versus-Host Disease

Another syndrome characterized by excessive production of cytokines, especially $\text{TNF-}\alpha$, is graft-versus-host disease. In this disease, described in more detail in [Chapter 29](#), grafted lymphocytes attack the tissues of the graft recipient. $\text{TNF-}\alpha$ from these cells causes mucosal destruction, leading to ulceration, diarrhea, and liver destruction.

4.7 PROTEIN MISFOLDING DISEASES

Amyloidosis is the name given to the deposition of insoluble proteins in tissues. These deposits appear as amorphous, eosinophilic, hyaline proteins in cells and tissues ([Figure 4-18](#)). Amyloid is produced as a result of errors in the folding of newly formed protein chains. These misfolded chains eventually aggregate to form insoluble fibrils. Electron microscopy shows that amyloid proteins consist of protein fibrils formed by peptide chains cross-linked to form β -pleated sheets ([Figure 4-19](#)). This molecular conformation makes amyloid proteins extremely insoluble and almost totally resistant to proteases. Consequently, once deposited in cells or tissues, amyloid deposits are almost impossible to remove. Amyloid infiltration eventually leads to gradual cell loss, tissue destruction, and death. Amyloidosis may be systemic (involving multiple organs) or localized (involving only one organ).

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Many different proteins can misfold and so form amyloid. For example, amyloidosis may develop when infections or inflammation cause a sharp rise in the concentration of the acute-phase protein SAA. A 76-residue proteolytic fragment of SAA can accumulate, misfold, aggregate, and be deposited in multiple organs. This material, one of the most common forms in domestic animals, is called reactive amyloid. Reactive amyloidosis is associated with chronic inflammation in diseases such as mastitis, osteomyelitis, abscesses, traumatic pericarditis, and tuberculosis

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FIGURE 4-19 Amyloid fibrils. An electron micrograph showing bundles of paired amyloid fibrils deposited parallel to a cell membrane. (Courtesy Dr. E.C. Franklin. From Franklin EC: Adv Immunol 15:25, 1972.)

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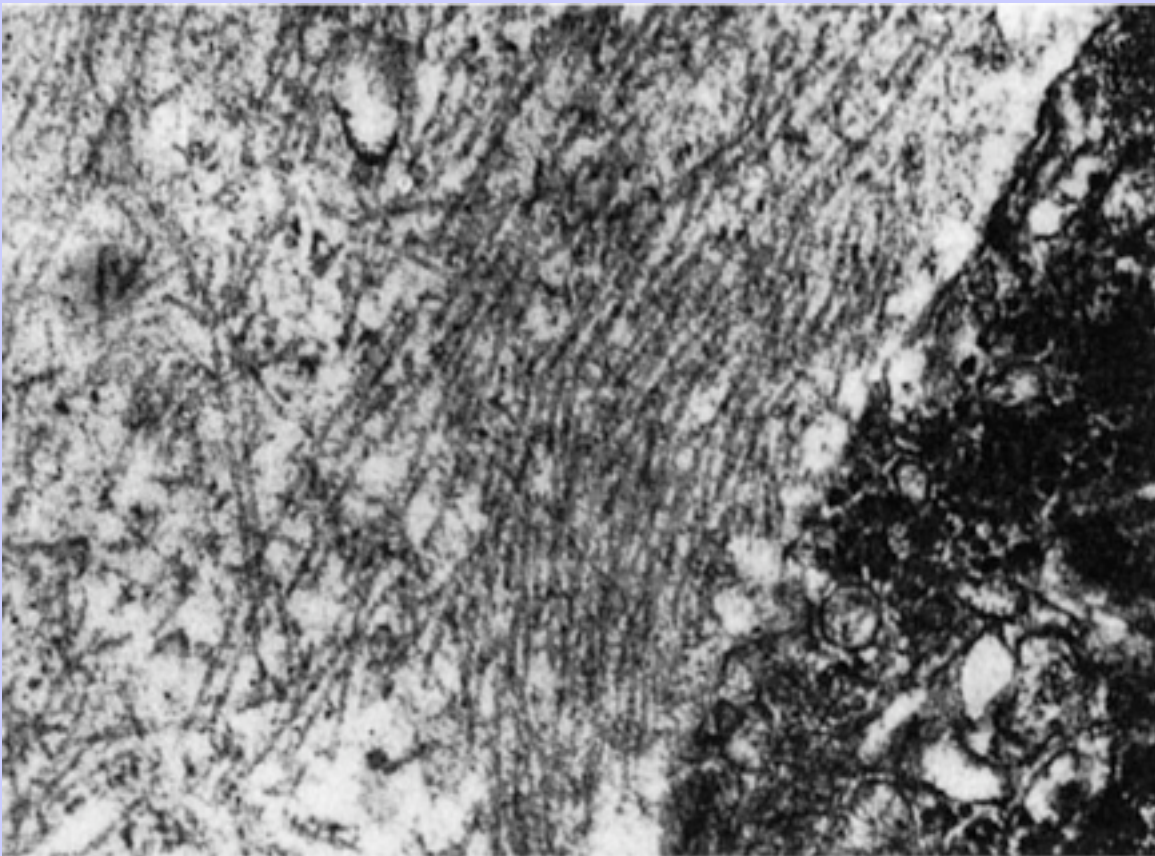
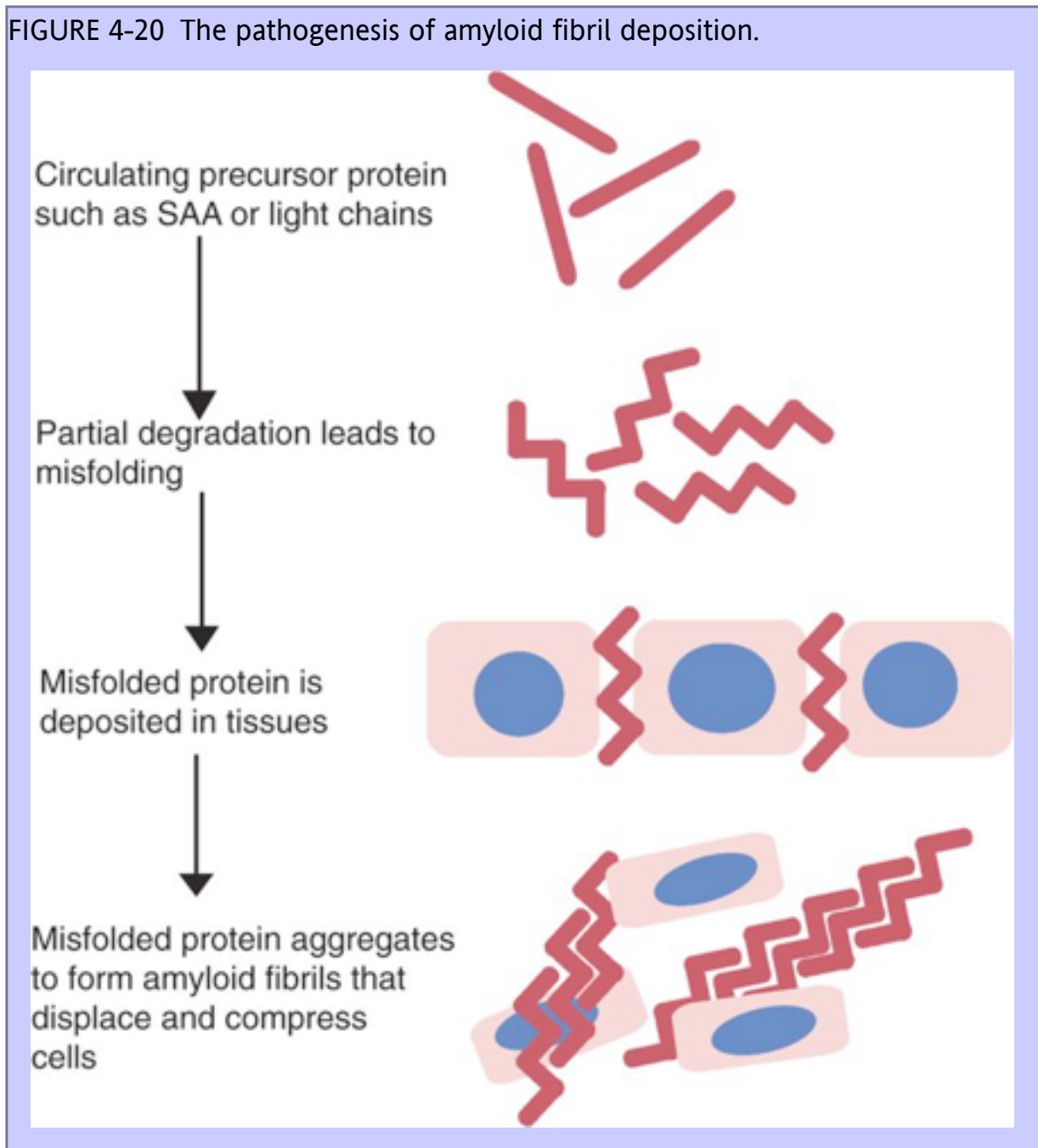


FIGURE 4-20 The pathogenesis of amyloid fibril deposition.



(Figure 4-20). Reactive amyloidosis is a major cause of death in animals repeatedly immunized for commercial antiserum production. Familial amyloidosis of Shar-Pei dogs consists of reactive amyloid deposited following chronic immune-mediated arthritis.

Multiple myelomas are plasma cell tumors that secrete antibodies, especially antibody light chains (see [Chapter 13](#)). Their presence leads to the production of huge quantities of antibody light chains and their fragments. The misfolding of these light chains and their fragments results in the deposition of immunogenic amyloid (AL). Although AL amyloid is the most common form of amyloid in humans, it is very rare in domestic animals.

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Several forms of localized amyloidosis are recognized in domestic animals. For example, old dogs may suffer from vascular amyloidosis, in which amyloid is deposited in the media of leptomeningeal and cortical arteries. An inherited form of amyloid has been described in Abyssinian cats. Tumorlike amyloid nodules and subcutaneous amyloid have been reported in horses. In general, however, amyloid deposits are found in the liver, spleen, and kidneys, particularly within glomeruli. In humans, amyloid fibrils are deposited in the neurons of patients with Alzheimer's disease. Misfolded prion proteins appear to be the cause of spongiform encephalopathies such as “mad-cow” disease. Prions, the infectious proteins responsible for spongiform encephalopathies, are protease-resistant forms of a cellular protein, PrP^c, that is important for normal macrophage functions. These prion proteins play a role in resistance to intracellular bacteria such as *Brucella*.

It is of interest to note that even reactive amyloidosis is somewhat “transmissible,” since inoculation of AA proteins into an animal will hasten the development of amyloidosis. They seem to act by providing a substrate upon which other misfolded proteins can be deposited. Similarly, silk fibers formed from a protein composed of β -sheets may also trigger amyloidosis when injected into mice.

4.8

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5 CHAPTER 5 The Complement System

5.1 KEY POINTS

- The complement system is a major component of both the innate and acquired immune systems.
- Complement proteins are found in normal serum.
- The complement system is activated by two innate pathways: the alternative pathway and the lectin pathway.
- The complement system is activated by antibodies bound to antigen—the classical pathway.
- Complement components, especially C3b, bind covalently to invading microbes and so opsonize them.
- Complement components may form a membrane-attack complex and punch holes in microbes.
- The complement system plays a key role in triggering inflammation through the release of the potent chemoattractant C5a.
- Deficiencies of some complement components lead to increased susceptibility to infections.

Protection from infection requires an immediate response by the innate immune system. A very important component of this response is the complement system. The complement system is a defense mechanism activated by both innate and acquired immune mechanisms. It consists of many different serum proteins together with an associated group of cell membrane proteins. These proteins have inflammatory, protective, and immunoregulatory functions ([Figure 5-1](#)).

Complement proteins act through enzymic pathways that cause specific proteins to bind covalently (and hence irreversibly) to the surface of invading microbes. Once bound, these proteins can destroy the invaders. In healthy, uninfected animals these pathways are inactive. However, they can be activated either by the presence of antibodies on the surface of an organism or simply by the presence of the complex carbohydrates found on the surface of infectious agents. Because the complement system is so potent, it must be carefully regulated and controlled. This in turn makes for significant complexity.

The complement system can be activated by at least three different pathways, referred to as the alternative, the lectin, and the classical pathways ([Figure 5-2](#)). The alternative and lectin pathways are activated directly by microbial carbohydrates—typical examples of the pathogen-associated molecular patterns that trigger innate immunity. The classical pathway, in contrast, is an evolutionary recent pathway activated by antibodies bound to the surface of an organism and thus works only in association with acquired immune responses.

5.2 COMPLEMENT PROTEINS

The proteins that form the complement system are either labeled numerically with the prefix C (e.g., C1, C2, C3) or designated by letters of the alphabet (B, D,

FIGURE 5-1 The functions of the complement system. Complement may either alter microbial membranes or alternatively trigger inflammation. Either way, it hastens the elimination of microbial invaders and is thus a key component of the innate immune system.

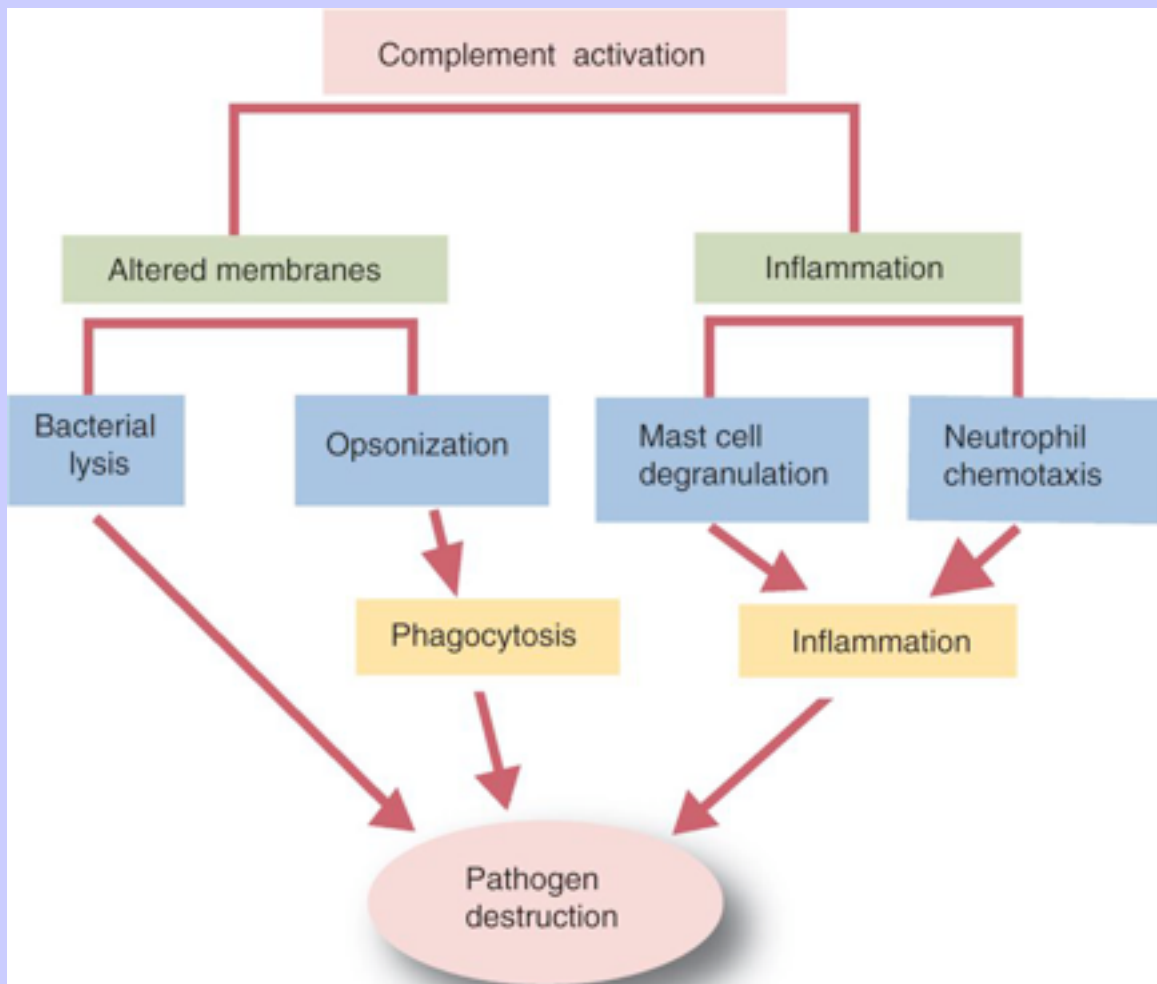
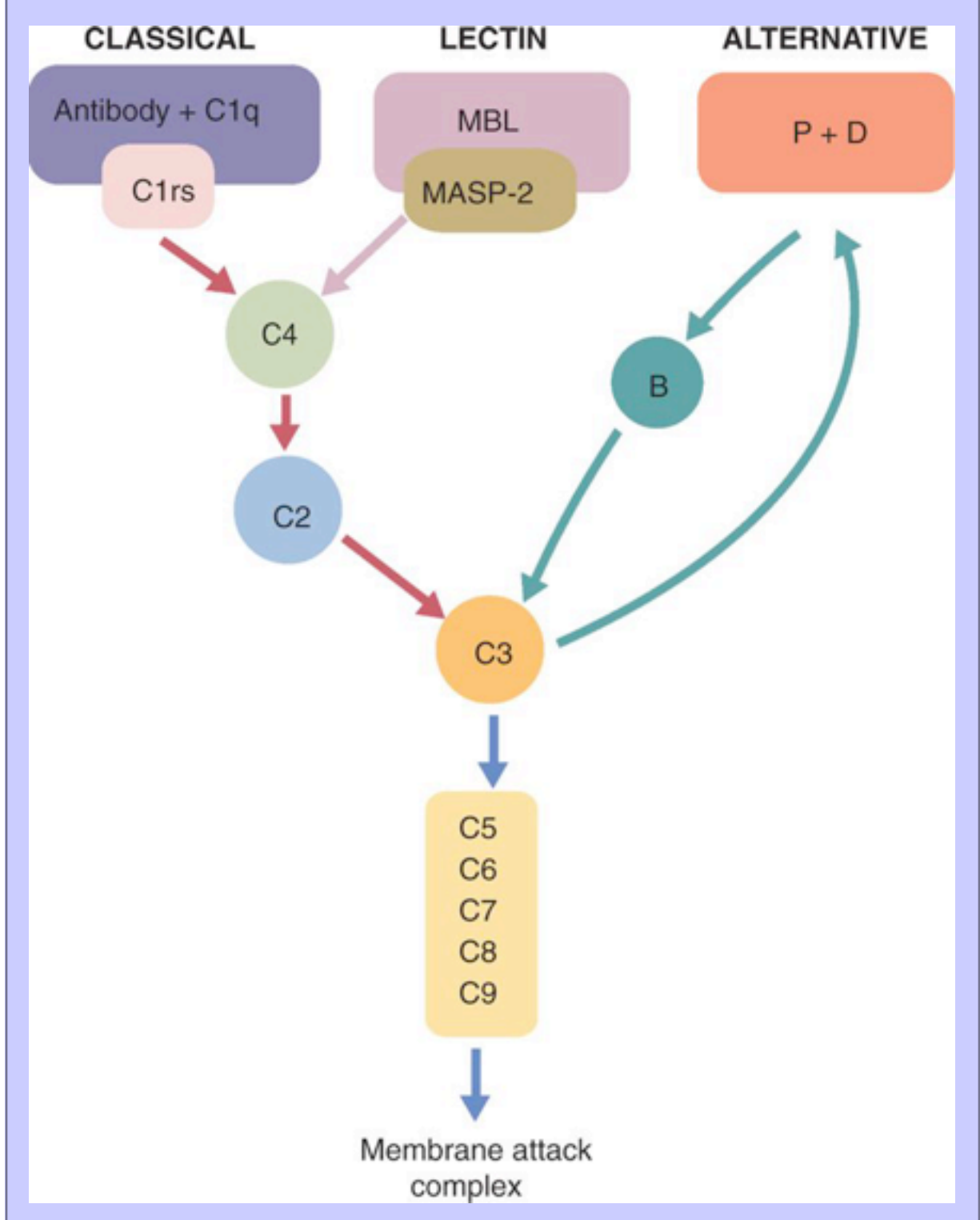


FIGURE 5-2 The three pathways by which the complement system can be activated.



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P, and so forth). There are at least 30 such proteins. Some are found free in serum; others are cell-bound receptors. Complement proteins account for about 15% of the globulin fraction of serum. The molecular weights of complement components vary from 24 kDa for factor D to 460 kDa for C1q. Their serum concentrations in humans vary between 20 mg/ml of C2 and 1300 mg/ml of C3 ([Table 5-1](#)). Complement components are synthesized at various sites throughout the body. Most C3, C6, C8, and B are made in the liver, whereas C2, C3, C4, C5, B, D, P, and I are made by macrophages. Neutrophils can store large quantities of C6 and C7. As a result, these components are readily available for defense at sites where macrophages and neutrophils accumulate.

5.3

ACTIVATION PATHWAYS

5.3.1

The Alternative Pathway

The alternative pathway of complement activation is an evolutionary ancient pathway that is even found in some invertebrates. It is triggered when microbial cell walls come into contact with complement components in the bloodstream and thus is a key component of innate immunity.

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The most important complement protein is called C3. C3 is a disulfide-linked heterodimer with α and β chains. It is synthesized by liver cells and macrophages and is the complement component of highest concentration in serum.

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Table 5-1 Complement Components

Name	MW (kDa)	Serum conc. (µg/ml)
Classical Pathway		
C1q	460	80
C1r	83	50
C1s	83	50
C4	200	600
C2	102	20
C3	185	1300
Alternate Pathway		
D	24	1
B	90	210
Terminal Components		
C5	204	70
C6	120	65
C7	120	55
C8	160	55
C9	70	60
Control Proteins		
C1-INH	105	200
C4BP	550	250
H	150	480
I	88	35
Ana INH	310	35
P	4 × 56	20
S	83	500

C3 possesses a hidden thioester chemical group. This is a highly reactive group that, when activated, binds to acceptor groups on many pathogens and marks them for destruction by immune cells. Unfortunately, similar acceptor groups are found on many normal tissues. Thus the activation of the thioester group must be very carefully regulated to ensure that the complement system does not attack normal tissues. In unactivated C3 the thioester group is kept hidden inside the folded molecule. In healthy normal animals, C3 breaks down slowly but spontaneously into two fragments called C3a and C3b ([Figure 5-3](#)). This breakdown opens up C3b to reveal the thioester group that then generates a reactive carbonyl group. This highly reactive carbonyl group then irreversibly binds the C3b to nearby surfaces ([Figure 5-4](#)). It also exposes binding sites for factor H. When factor H binds to these sites, a protease called factor I cleaves the C3b, shutting off further activity and producing iC3b

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and C3c. iC3b is the ligand of receptors found on circulating leukocytes ([Figure 5-5](#)). It stimulates these cells to engulf pathogens and activate inflammatory cells. The final breakdown product, C3dg, targets pathogens to surface receptors on B cells and so promotes antibody production. Thus C3b is destroyed immediately after being deposited on a nearby surface. This destruction depends on the activity of factor H, which depends in turn on the nature of the target surface. When factor H interacts with normal cell

FIGURE 5-3 The alternative complement pathway. Surface-bound C3b may either be destroyed, as normally happens, or be activated by the presence of an activating surface.

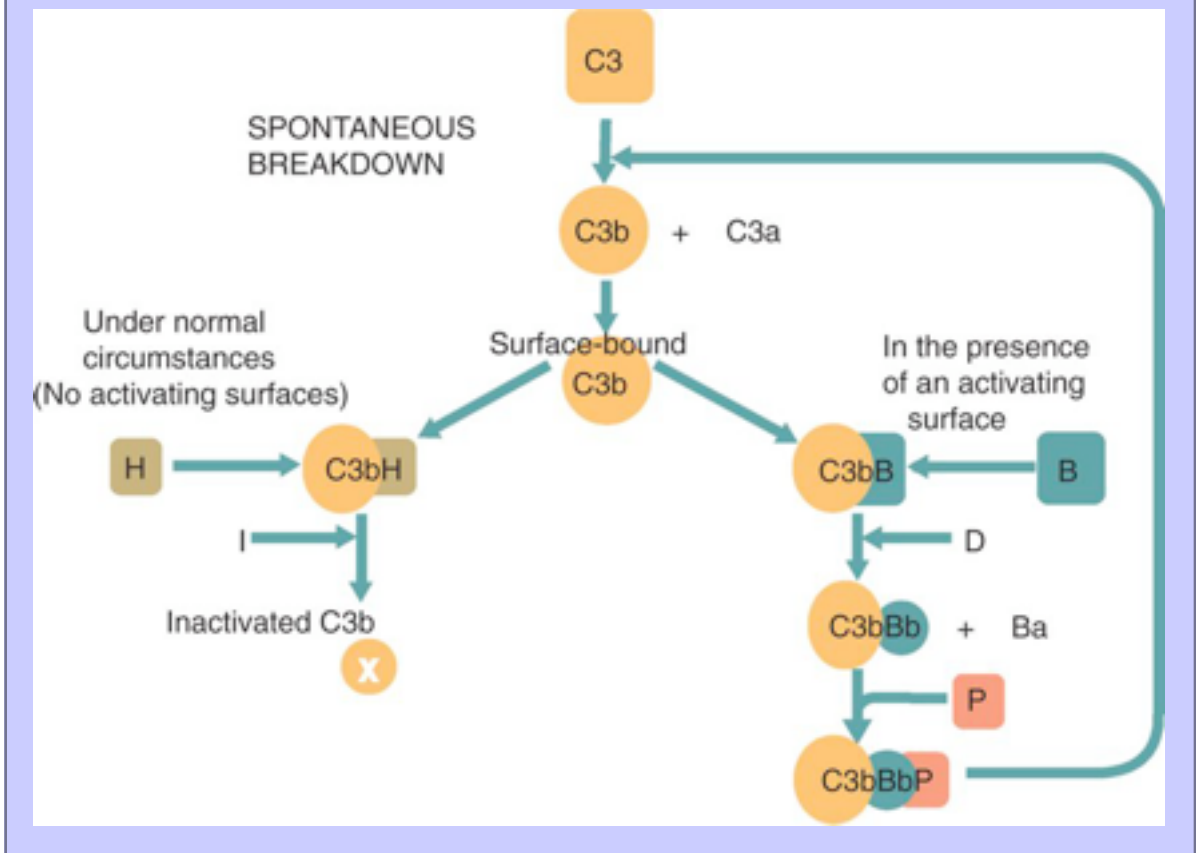


FIGURE 5-4 Activation of C3 involves its cleavage by C3 convertase. This exposes a thioester bond between a cysteine and a glutamine. This bond breaks to form a reactive carbonyl group that enables the molecule to bind covalently (and hence irreversibly) to target cell surfaces. Removal of C3a also reveals the binding sites for factor H and factor B.

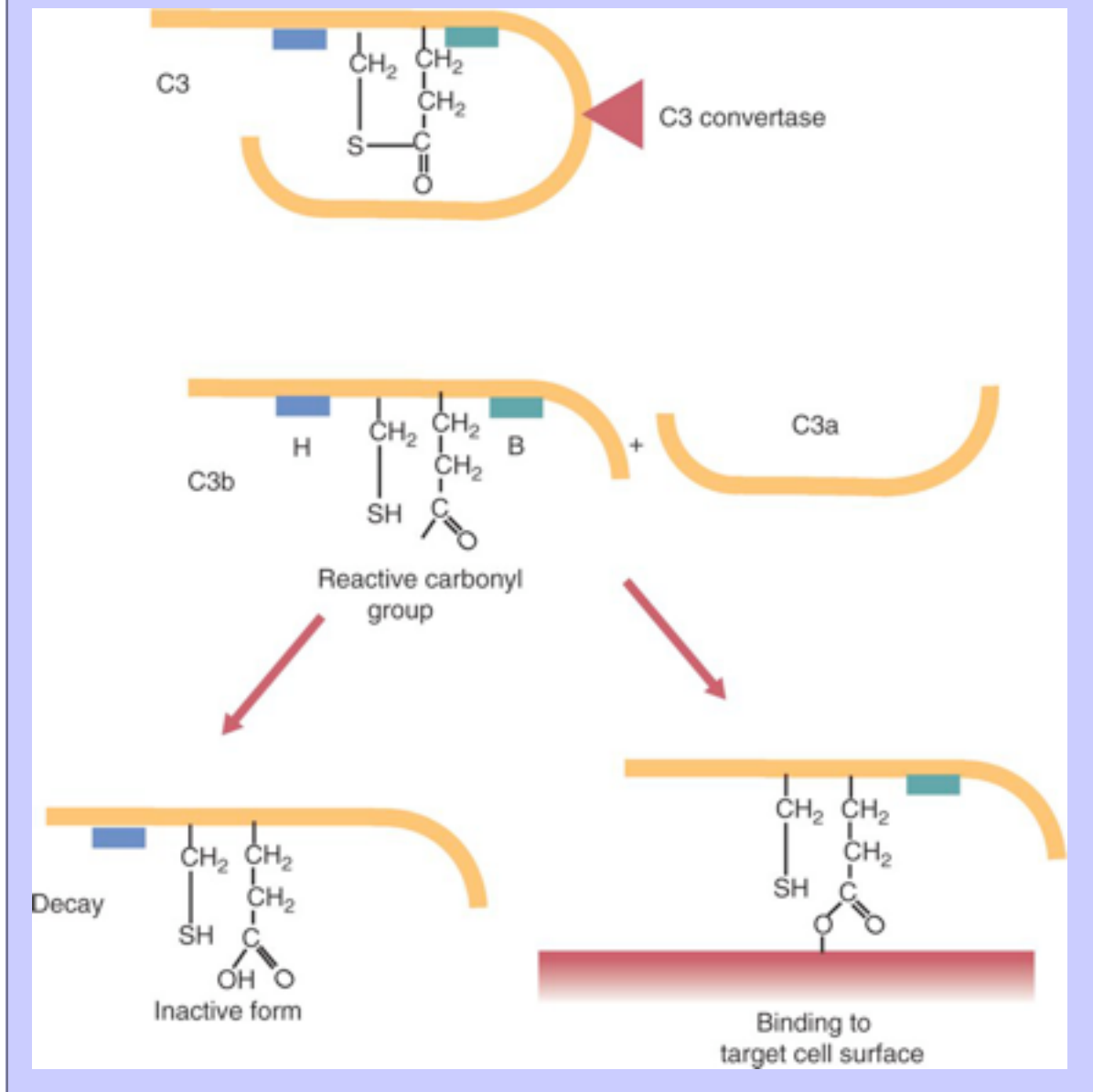
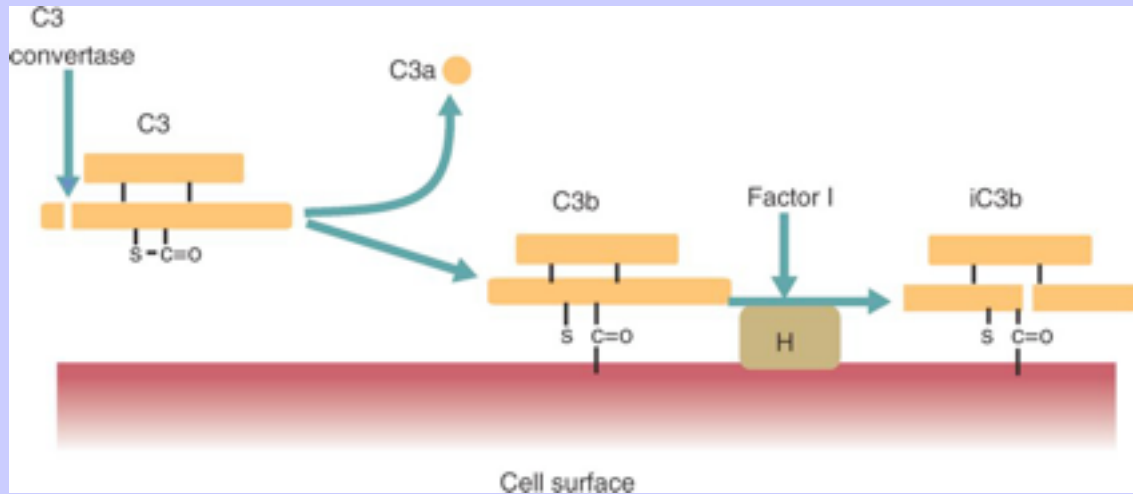


FIGURE 5-5 Activated C3 binds to a cell surface. This C3b is normally inactivated by the actions of factors H and I. However, factor H must first be activated by binding to the surface. In the absence of factor H, factor I will not work. In this case C3b persists and activates the terminal complement pathway.



surfaces, glycoproteins rich in sialic acid and other neutral or anionic polysaccharides enhance its binding to C3b, factor I is activated, and the C3b is destroyed. Thus in a healthy individual, factors H and I destroy C3b as fast as it is generated. On the other hand, on bacterial cell walls, lipopolysaccharides and other carbohydrates lack sialic acid. As a result, factor H cannot bind to C3b, factor I is inactivated, and the C3b persists.

The opening up of C3b also exposes binding sites for another complement protein called factor B to form a complex called C3bB. The bound factor B is then cleaved by a protease called factor D, releasing a soluble fragment called Ba and leaving C3bBb attached to the bacterial wall. This bound C3bBb is a potent protease whose preferred substrate is C3. (It is therefore called the alternative C3 convertase.) Factor D can act only on factor B after it is bound to C3b. This constraint is called substrate modulation, and it occurs at several points in the complement pathways. It presumably ensures that the activities of enzymes such as factor D are confined to the correct molecules.

The alternative C3 convertase, C3bBb, can split C3 and so generate more C3b. However, C3bBb has a half-life of only 5 minutes. If another protein called factor P (or properdin) binds to the complex to form C3bBbP, its half-life is extended to 30 minutes. Since C3b thus serves to generate more C3bBbP, the net effect of all this is that a positive loop is generated where increasing amounts of C3b are irreversibly bound to the surface of the invading organism.

Surface-bound C3b also binds another protein called C5 (Figure 5-6). Once C5 is bound to C3b, substrate modulation occurs and the C5 can also be cleaved by C3bBb (Figure 5-7). This enzyme splits off a small peptide called C5a, leaving a large fragment, C5b, attached to the C3b. This cleavage also exposes a site on C5b that can bind two new proteins, C6 and C7, to form a multimolecular complex called C5b67 (Figure 5-8). The C5b67 complex can then insert itself into the microbial cell membrane. Once inserted in the surface of an organism, the complex will bind a molecule of C8. Twelve to 18 C9 molecules then aggregate with the C5b678 complex to

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form a tubular structure called the membrane attack complex (MAC). The MAC inserts itself into a microbial cell membrane and effectively punches a hole in the invader. If sufficient MACs are formed on an organism, it will be killed by osmotic lysis. These MACs can be seen by electron microscopy as ring-shaped structures on the microbial surface with a central electron-dense area surrounded by a lighter ring of poly C9 ([Figure 5-9](#)).

5.3.2

The Lectin Pathway

The second method of activating the complement system involves the binding of microbial carbohydrates to serum lectins. These bound lectins then activate proteases that trigger complement activation. Like the alternative pathway, this is an innate defense mechanism triggered simply by the presence of bacterial cell walls within the bloodstream ([Figure 5-10](#)).

Mannose-binding lectin (MBL) in serum can bind to mannose or N-acetylglucosamine on microbial cell

FIGURE 5-6 The two C3 convertases, C4b2b and C3bBb, act on C5 when it is linked to C3b and cleave off a small peptide called C5a. In so doing they reveal a site that binds C6 and C7.

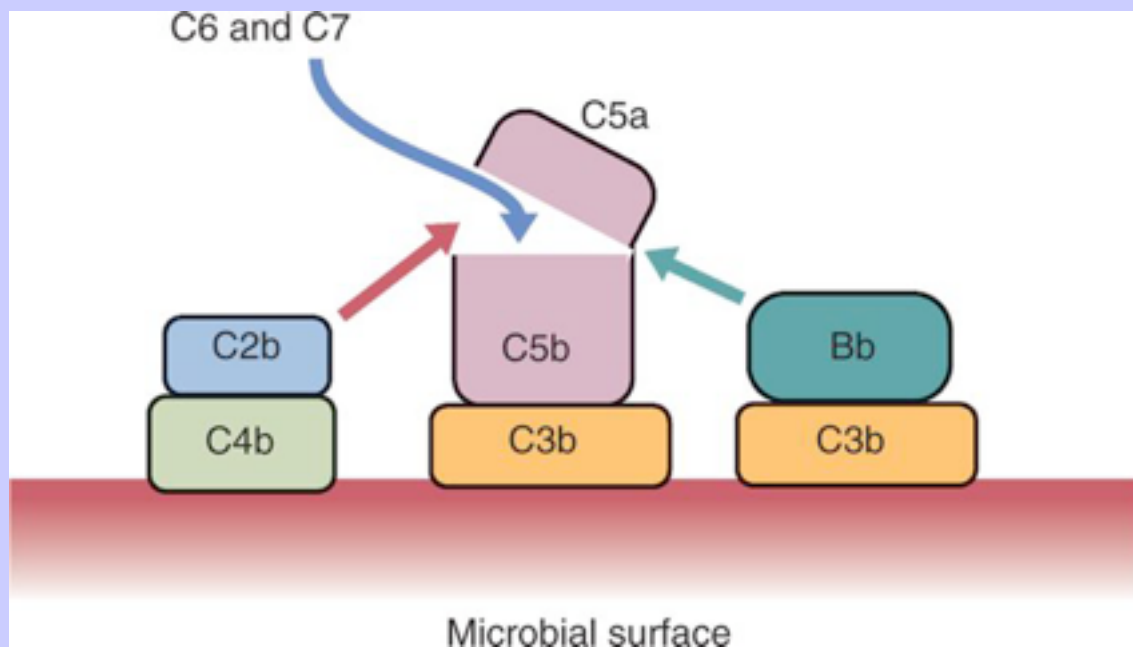
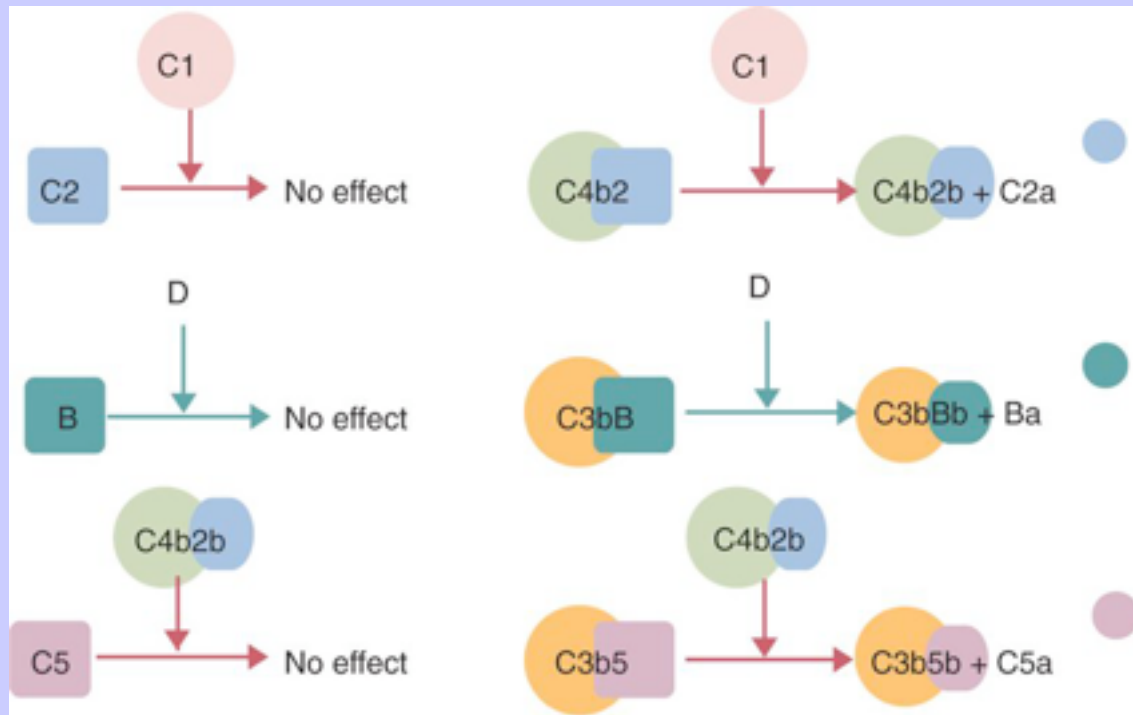


FIGURE 5-7 Substrate modulation is one way in which the complement system is regulated. The target for a protease cannot be cleaved unless it is first bound to another protein. Examples of substrate modulation include the cleavage of factors C2, B, and C5 only after they have bound to C4, C3, and C3, respectively.



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FIGURE 5-8 The terminal complement pathway. The progressive aggregation of the terminal complement components eventually leads to the polymerization of C9 and the assembly of a membrane attack complex.

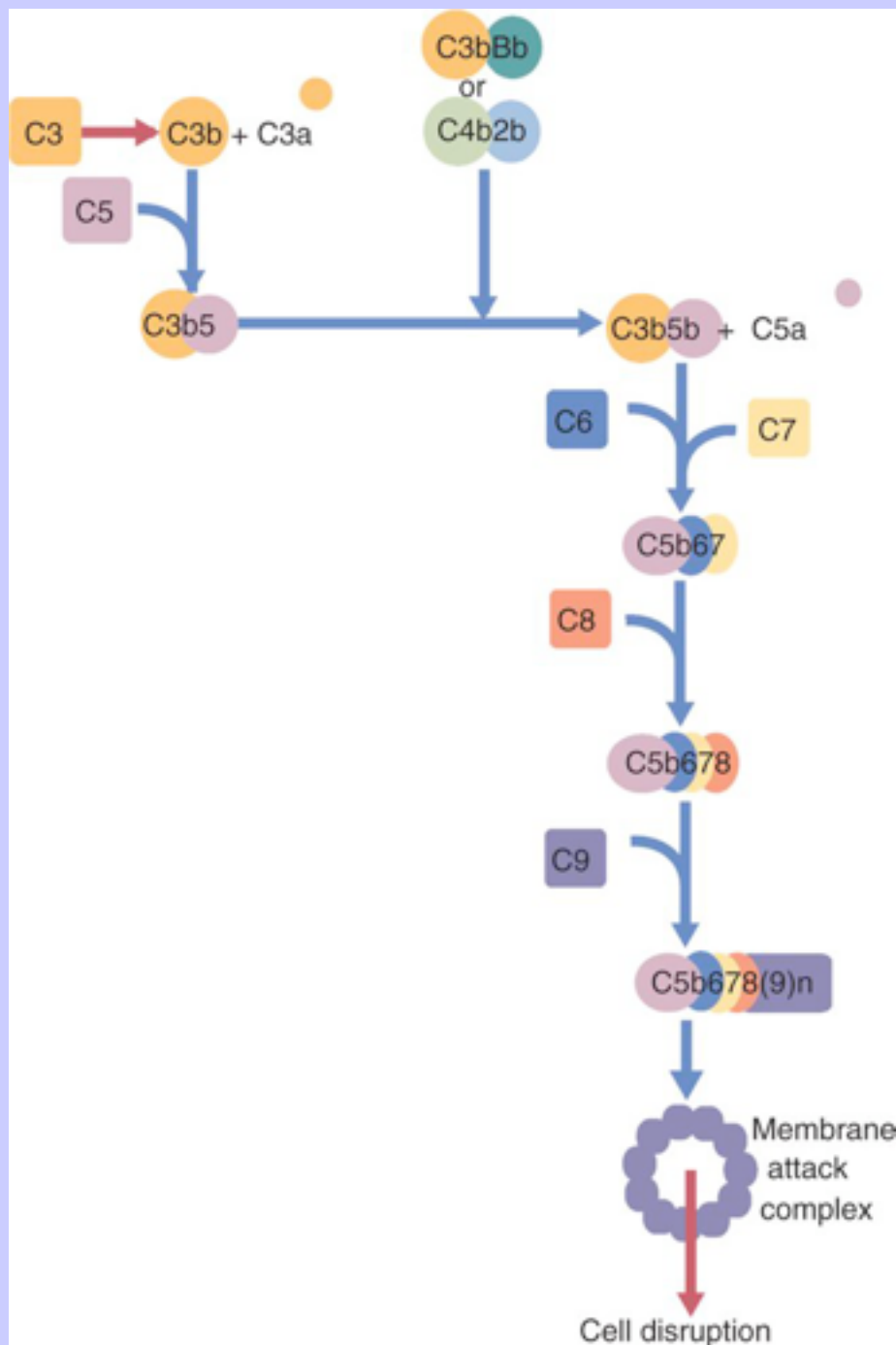
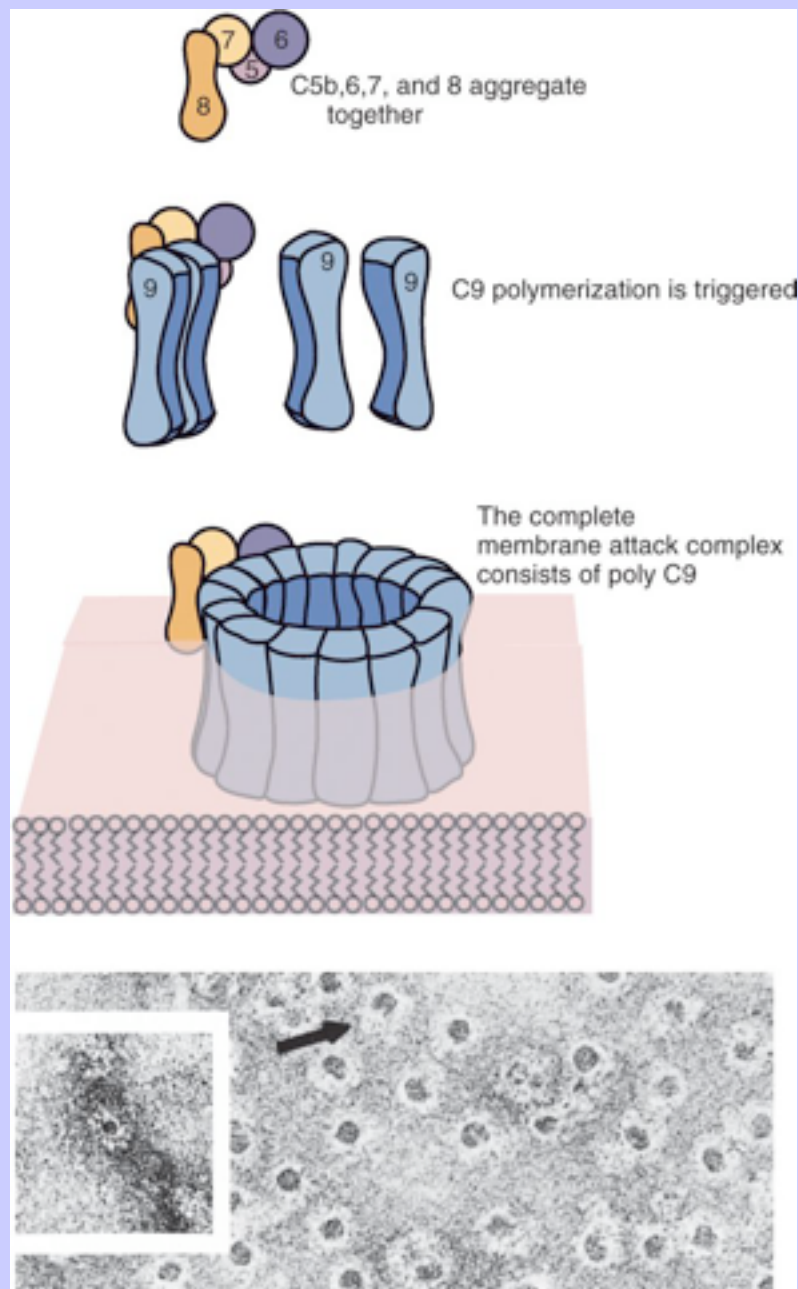


FIGURE 5-9 Formation of poly C9 by the terminal complement pathway and an electron micrograph of poly C9 complement lesions on an erythrocyte membrane. The insert shows a mouse complement lesion. The arrow points to a possible C5b678 complex. Compare these lesions to the T cell polyperforins in [Figure 17-9](#). (From Podack ER, Dennert G: *Nature* 307:442, 1983.)



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walls. (Carbohydrates such as galactose or sialic acid found on mammalian glycoproteins do not bind MBL.) Thus MBL can bind to the surface of bacteria, fungi, parasitic protozoa, and viruses (see [Chapter 2](#)). Ficolins are another family of lectins that can activate the lectin pathway through MBL-associated serine proteases (MASPs).

Once it has bound to microbial surfaces, the MBL will bind and then activate the serum protease MASP-2. It is believed that binding of MBL to carbohydrates on the microbial surface results in conformational changes that activate MASP-2. Activated MASP-2, in turn, acts on the protein C4, splitting it into C4a and C4b. Removal of C4a exposes a thioester group on the C4b and generates a reactive carbonyl group that covalently attaches the C4b to the microbial surface (see [Figure 5-4](#)). C2 is a glycoprotein that binds to C4b to form a complex, C4b2. C2 is then also cleaved by MASP-2 to generate C4b2b.

Cell-bound C4b2b acts on the a chain of C3 to generate C3a and C3b. As in the activation of C4, C3 exposes its thioester group when C3a is split off. As a result, C3b molecules also bind covalently to surfaces carrying C4b2b. The activation of C3b by C4b2b is a major step because each C4b2b complex can activate as many as 200 C3 molecules, which are then irreversibly attached to nearby surfaces. Since the reactions of the complement system are usually confined to the microenvironment close to microbial surfaces, C3 will bind to these organisms. The bound C3b can bind C5 and cleave it to C5a and C5b. The complement pathway then can proceed to completion, causing the destruction of the organism by MACs as described above.

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FIGURE 5-10 Complement activation by the lectin pathway.

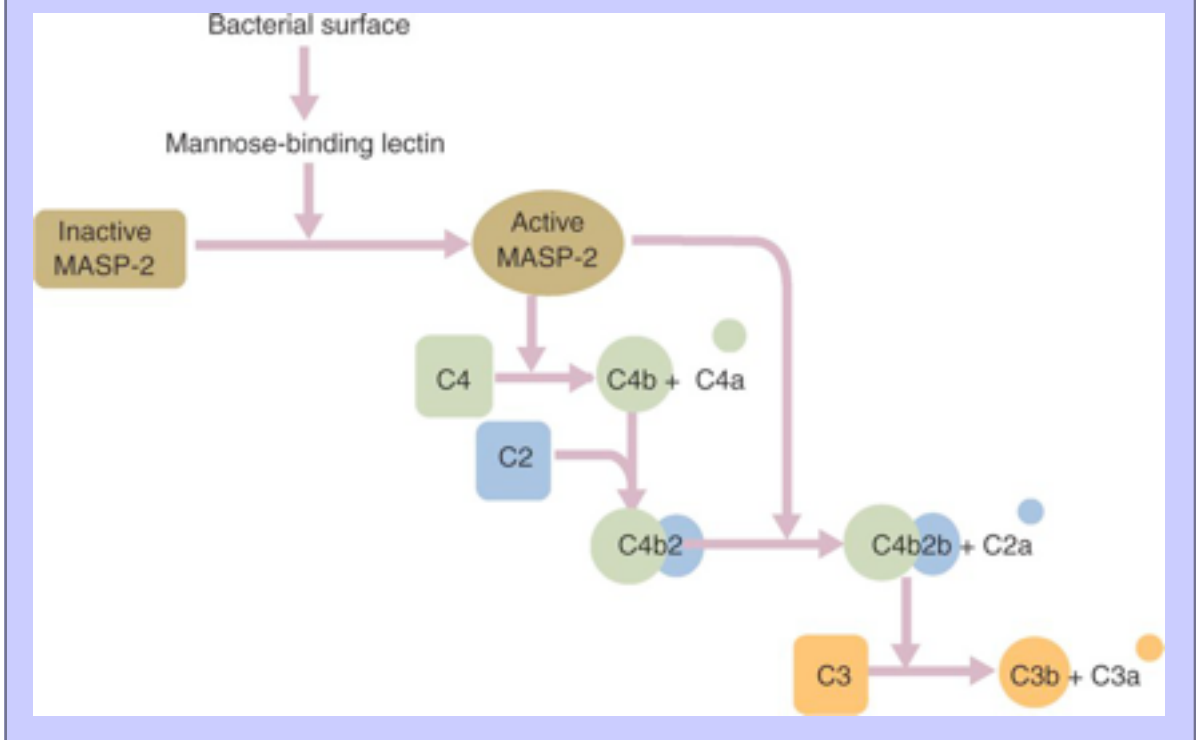
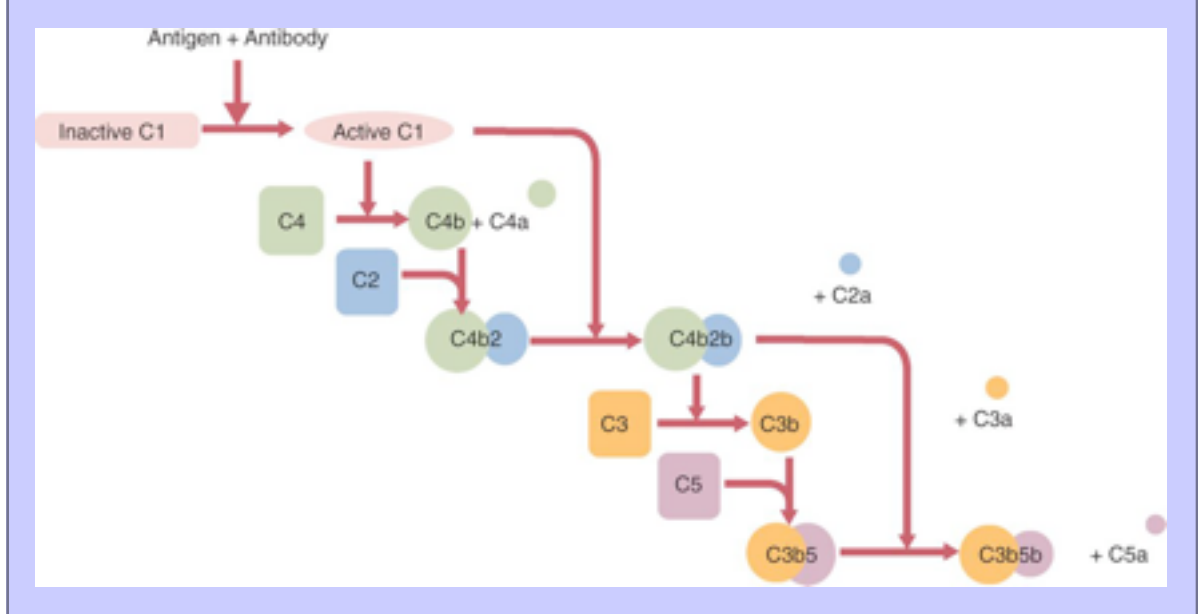


FIGURE 5-11 The basic features of the classical complement pathway.



The MBL–MASP-2 pathway is ancient, having existed for at least 300 million years. Although in many ways it duplicates the alternative pathway, it provides yet another example of duplication of mechanisms to “guarantee” protection.

5.3.3

The Classical Pathway

The classical complement pathway ([Figure 5-11](#)) is usually triggered by antibodies bound to the surface of a foreign organism. It is thus part of the acquired immune system. Because of this, it cannot be triggered until antibodies are made, which may occur as late as 7 to 10 days after infection. Nevertheless, once activated it is a very efficient complement-activating pathway. When antibody molecules bind to an antigen, they change their molecular shape and expose active sites on their Fc regions. If several antibody molecules are bound to an organism, multiple active sites will be exposed within a small area. These multiple active sites trigger classical complement pathway activation.

The first component of the classical complement pathway is a multimolecular protein complex called C1. C1 consists of three proteins (C1q, C1r, and C1s) bound together by calcium. C1q looks like a six-stranded whip when viewed by electron microscopy ([Figure 5-12](#)). Two molecules of C1r and two of C1s form a figure-of-eight structure located between the C1q strands. C1q is activated when the tips

FIGURE 5-12 The structure of C1 and its role in interacting with antibodies to initiate the classical complement pathway.

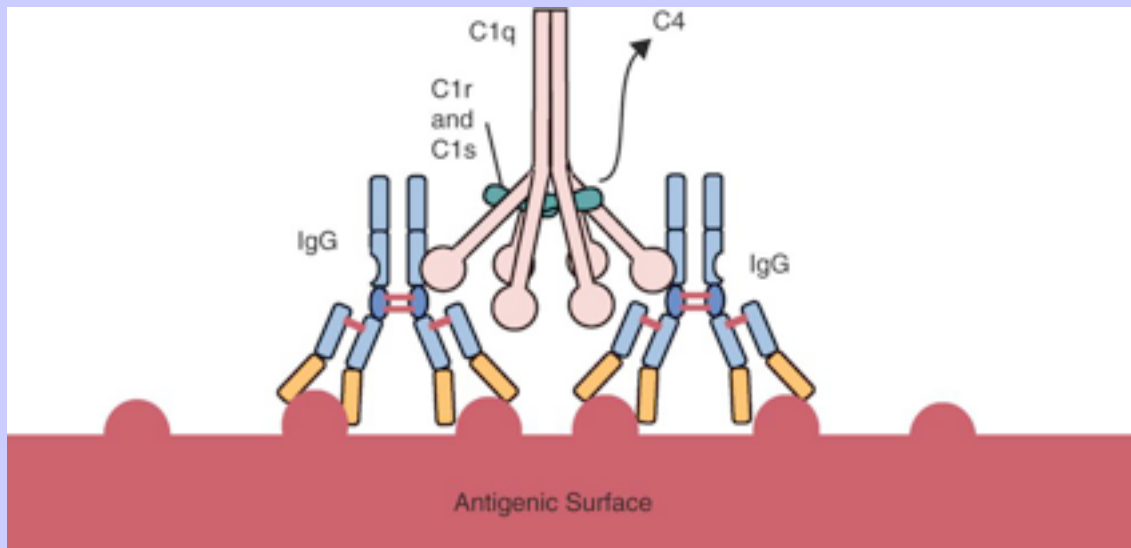
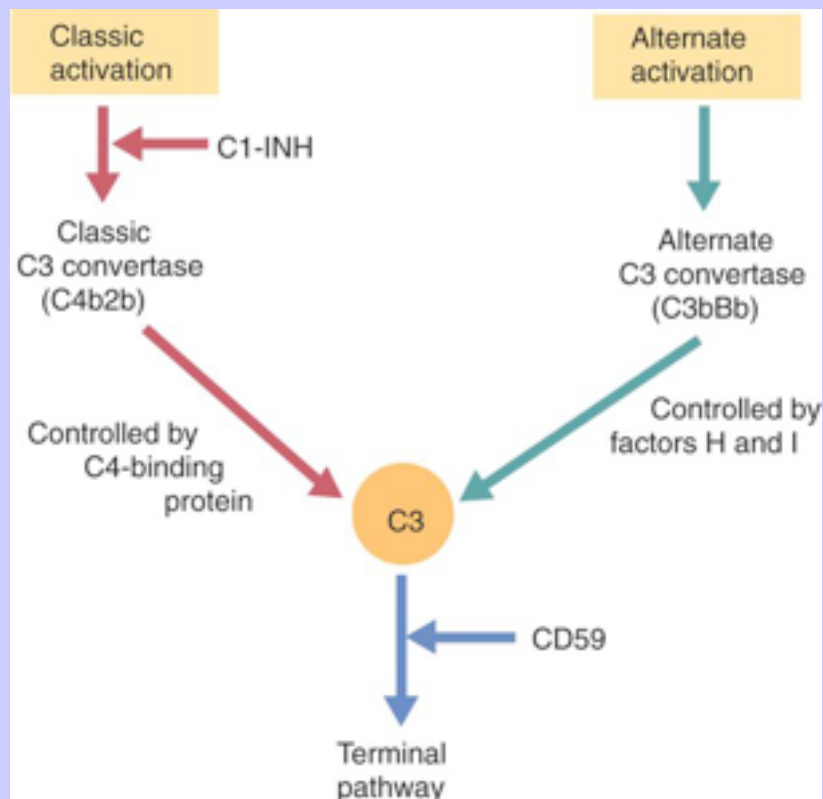


FIGURE 5-13 Basic control mechanisms of the complement system.



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of at least two strands bind to complement-activating sites on immunoglobulin Fc regions. Binding to the immunoglobulin causes a conformational change in C1q that is transmitted to C1r. As a result, C1r reveals an active proteolytic site that cleaves a peptide bond in C1s to convert that molecule to an enzymatically active form. Single antigen-bound molecules of immunoglobulin M (IgM) or paired antigen-bound molecules of IgG are needed to activate C1. The polymeric IgM structure readily provides two closely spaced complement-activating sites. In contrast, two IgG molecules must be located very close together to have the same effect. As a result, IgG is much less efficient than IgM in activating the classical pathway. C1 may also be activated directly by some viruses or by bacteria such as *Escherichia coli* and *Klebsiella pneumoniae*.

Activated C1s cleaves C4 into C4a and C4b. C2 then binds to C4b to form C4b2. Activated C1s then splits the bound C2 generating a small peptide, C2a, and C4b2b. C1s cannot act on soluble C2; the C2 must first be bound to C4b before it can be cleaved (another example of substrate modulation). The C4b2b complex, as described above, is a potent protease that cleaves C3 and is therefore called classical C3 convertase. C3b generated in this way binds and activates C5. Subsequent reactions lead to formation of the MAC and microbial killing.

The close relationship between the lectin pathway and the classical pathway is shown by the observation that some lectins can also activate the classical pathway. For example, a lectin called specific intracellular adhesion molecule-grabbing nonintegrin (SIGN)-R1 found on the surface of macrophages binds to bacteria such as *Streptococcus pneumoniae* and thus acquires the ability to activate C1 and trigger the classical pathway directly.

5.4 REGULATION OF COMPLEMENT

The consequences of complement activation are so significant and potentially dangerous that all of the activation pathways must be carefully controlled by regulatory proteins ([Figure 5-13](#)).

The most important regulator of the classical pathway is C1-inactivator (C1-INH). C1-INH blocks the activities of active C1r and C1s. Other regulatory proteins control the activities of the C3 and C5 convertases. For example, CD55, or decay accelerating factor, is a glycoprotein expressed on the surface of red blood cells, neutrophils, lymphocytes, monocytes, platelets, and endothelial cells. CD55 binds to the convertases and accelerates their decay. Its function is to protect normal cells from complement attack. Other proteins that accelerate degradation of the convertases include factor H and C4-binding protein (C4BP) found in plasma and CD35 (CR1) and CD46 found on cell membranes. Control of the C5b6789 complex is mediated by three glycoproteins: vitronectin, clusterin, and, most importantly, CD59 (protectin). They all inhibit C5b678 insertion and C9 polymerization in normal cell membranes.

5.4.1 Complement Receptors

Five cell surface receptors for C3 or its fragments have been identified. These are called CR1 (CD35), CR2 (CD21), CR3 (CD11a/CD18), CR4 (CD11c/CD18), and CRIg.

CR1 binds C3b and C4b as well as the C3b breakdown product, iC3b. CR1 is found on primate red cells, neutrophils, eosinophils, monocytes, macrophages, B cells, and some T cells. Red cell CR1 accounts for 90% of all CR1 in the blood. In primates, CR1 removes immune complexes from the circulation. (Immune complexes bind to CR1 on red cells, and the coated red cells are then removed in the liver and spleen [see [Chapter 27](#)].) Deficiencies of complement components or their receptors may allow circulating immune complexes to accumulate in organs such as kidney and cause tissue damage. For example, some patients with the autoimmune disease systemic lupus erythematosus have a CR1 deficiency and are thus unable to remove these immune complexes effectively. C3-deficient dogs develop immune complex-mediated kidney lesions for the same reason.

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CR2 (CD21), found on most B cells, binds a breakdown fragment of C3 called C3d. This cell surface receptor forms a complex with CD19 that regulates B cell responses (see [Chapter 13](#), [Figure 13-10](#)). To respond optimally to antigens, B cells require stimulation by C3d acting through CR2.

CR3 (CD11a/CD18) is an integrin that binds iC3b. It is found on macrophages, neutrophils, and natural killer cells. A genetic deficiency of CR3 (leukocyte adherence deficiency) has been described in humans, cattle, and dogs in which affected individuals experience severe recurrent infections (see [Chapter 34](#)).

CR4 (CD11c/CD18) is another integrin found on neutrophils, T cells, natural killer cells, a few platelets, and macrophages. It binds breakdown fragments of C3.

CR1g is expressed on tissue macrophages including Kupffer cells in the liver. It has an affinity for C3b and iC3b. It is a receptor for the C3-dependent opsonization of blood-borne pathogens.

5.5 OTHER CONSEQUENCES OF COMPLEMENT ACTIVATION

While microbial killing due to lysis mediated by MACs is the most obvious activity of the complement system, its protective effects go far beyond this, contributing to the body's defenses in many ways.

5.5.1 Opsonization

C3b and C4b bound covalently to a microbial surface effectively tag it as foreign and serve as very potent and effective opsonins. Phagocytic cells possess CR1, whereas tissue macrophages possess CR1g. Thus C3b-coated organisms will bind strongly to these cells and undergo type II phagocytosis (see [Chapter 3](#)). If for some reason these organisms cannot be ingested, then neutrophils may secrete their lysosomal enzymes and oxidants into the surrounding tissue fluid. These molecules then cause inflammation and tissue damage—a reaction classified as type III hypersensitivity (see [Chapter 27](#)).

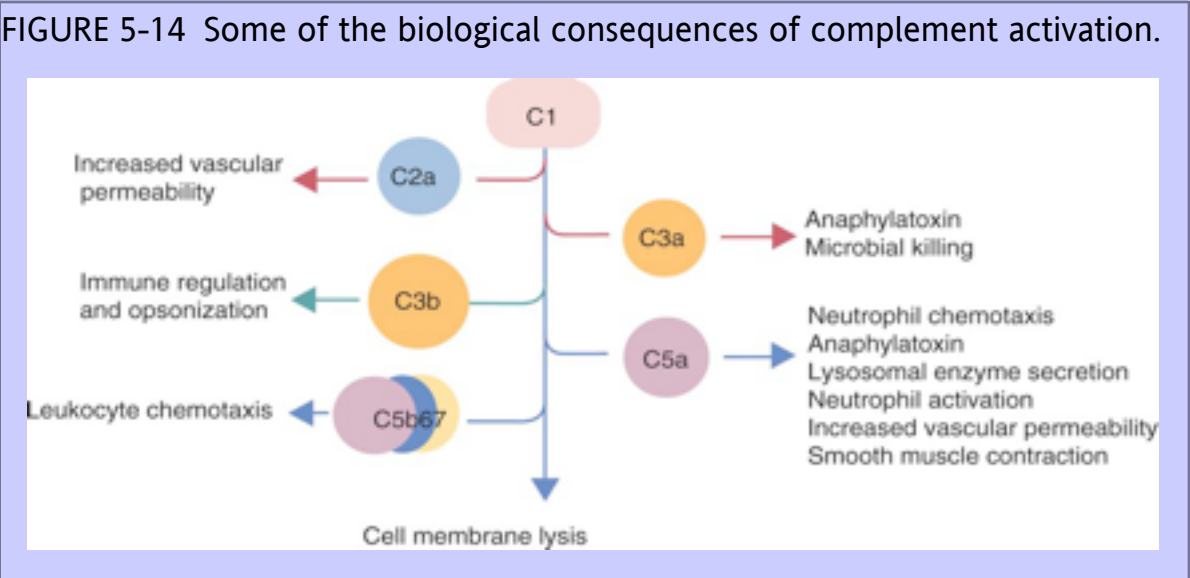
5.5.2 Chemotaxis

The complement system is a major contributor to acute inflammation. For example, activation of the complement system by any of its pathways generates several potent chemotactic peptides, including C5a and C5b67 ([Table 5-2](#)). C5b67 is chemotactic for neutrophils and eosinophils, whereas C5a attracts not only neutrophils and eosinophils but also macrophages and basophils. When C5a attracts neutrophils, it stimulates their respiratory burst and upregulates CR1 and integrin expression.

5.5.3 Inflammation

The small peptides C3a and C5a cause acute inflammation when injected into the skin. These molecules have been called anaphylatoxins because they degranulate mast cells and stimulate platelets to release the vasoactive molecules histamine and serotonin. They

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increase vascular permeability, causing lysosomal enzyme release from neutrophils and thromboxane release from macrophages (Figure 5-14). C3a and its inactivated derivative C3a-des Arg are also antibacterial peptides. Thus C3a is an efficient killer of *E. coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Streptococcus pyogenes*. The C3a appears to act by disrupting bacterial membranes. It therefore resembles the defensins and other antimicrobial peptides and provides yet another mechanism by which the complement system contributes to innate immunity.

Table 5-2 Complement-Derived Chemotactic Factors

Factor	Target
C3a	Eosinophils
C5a	Neutrophils, eosinophils, macrophages
C567	Neutrophils, eosinophils
Bb	Neutrophils
C3e	Promotes leukocytosis

5.5.4 Immune Regulation

Complement regulates antibody formation through C3d bound to antigen. When an antigen molecule binds to a B cell receptor, any C3d on its surface will bind to CD21/CD19 complexes on the B cell surface. (Remember that several hundred C3 molecules may attach to an antigen as a result of C3 convertase activity.) Activation of the CD21/CD19 complex sends a signal that significantly potentiates B cell receptor signaling and is an important co-stimulatory pathway for mature B cells. Thus depletion of C3 is associated with reduced primary antibody responses.

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Coating of antigens with C3d also permits antigens to bind to CR2 on dendritic cells and so influences antigen processing. In the absence of C3, immune complexes will not localize on follicular dendritic cells in germinal centers.

5.6 COMPLEMENT GENES

The genes coding for complement proteins are mainly scattered throughout the genome. However, two major gene clusters have been identified. The genes coding for C4, C2, and factor B are clustered within the major histocompatibility complex class III region; the genes for C4BP, CD55, CD35, CD21, CD46, and factor H are linked in the RCA (regulation of complement activation) cluster.

Complement components, like other proteins, may occur in different allelic forms. The precise number varies between components and species. For example, bovine factor H has three allotypes, equine C3 has six, and canine C3 has two. Canine C6 has seven allotypes, and porcine C6 has 14. Eleven allotypes of canine C7 have been identified, while canine C4 has at least five. There is an association among the C4-4 allotype, low serum C4 levels, and the development of autoimmune polyarthritis in dogs. Feline and equine C4 each have at least four allotypes.

5.7 COMPLEMENT DEFICIENCIES

5.7.1 Canine C3 Deficiency

Because the complement system is an essential defensive mechanism, complement deficiencies increase susceptibility to infections. The most severe of these diseases occurs in individuals deficient in C3. For example, a colony of Brittany Spaniels with an autosomal recessive C3 deficiency has been described ([Figure 5-15](#)). Dogs that are homozygous for this trait have no detectable C3, whereas heterozygous animals have C3 levels that are approximately half normal. Heterozygous animals are clinically normal. The homozygous-deficient animals have lower IgG levels than normal, and their ability to make antibodies against defined antigens is reduced. The dogs tend to make more IgM and less IgG. They experience recurrent sepsis, pneumonia, pyometra, and wound infections. The organisms involved include *Clostridium*, *Pseudomonas*, *E. coli*, and *Klebsiella*. Some affected dogs develop

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FIGURE 5-15 Inheritance of a C3 deficiency in a colony of Brittany Spaniels. The number below each circle or square represents the animal's C3 level as a percentage of a standard reference serum. The mean level in healthy spaniels was 126. (Squares denote males, circles denote females.) (From Winkelstein JA, Cork LC, Griffin DE, et al: *Science* 212:1169-1170, 1981.)

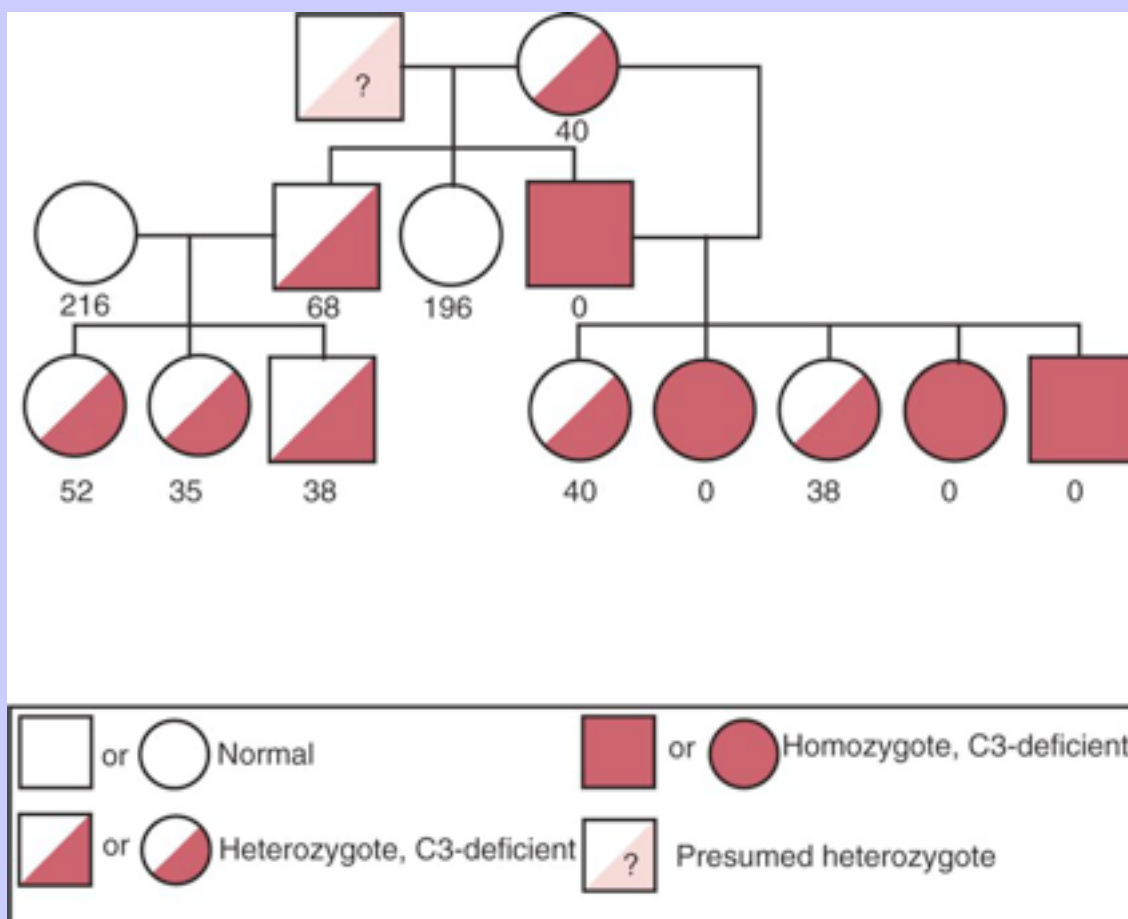
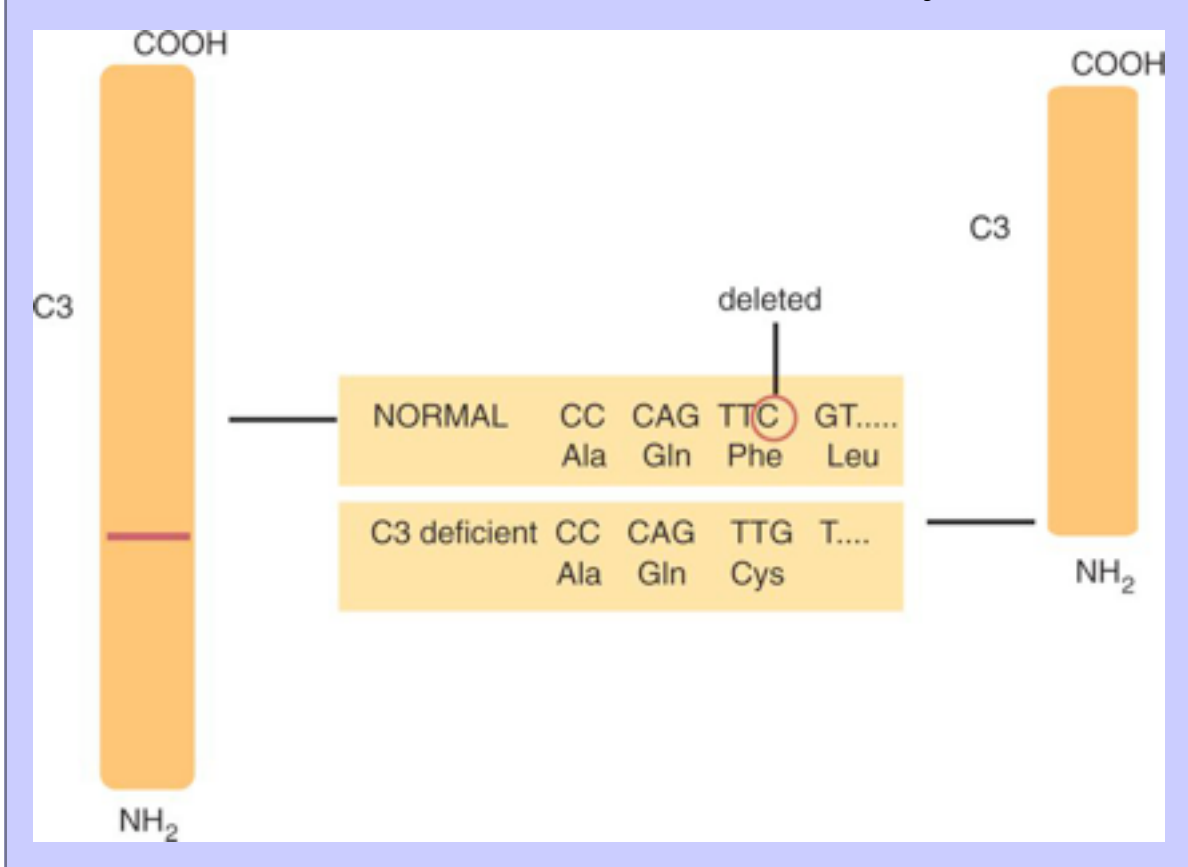


FIGURE 5-16 The mutation that results in canine C3 deficiency.



amyloidosis, and many develop an immune complex-mediated kidney disease (see [Chapter 27](#)). The mutation responsible for this deficiency (deletion of a cytosine residue) shortens the C3 chain as a result of a frameshift and the generation of a premature stop codon ([Figure 5-16](#)).

5.7.2

Porcine Factor H Deficiency

Factor H is a critical component of the alternative complement pathway. It normally inactivates C3b as soon as it is generated and so prevents excessive alternative pathway activation. If an animal fails to make factor H, C3b will be generated in an uncontrolled fashion. Factor H deficiency has been identified as an autosomal recessive trait in Yorkshire pigs. Affected piglets are healthy at birth and develop normally for a few weeks. However, eventually they fail to thrive, stop growing, become anemic, and die of renal failure.

On autopsy, multiple petechial hemorrhages are seen on the surface of the kidneys, accompanied by

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FIGURE 5-17 **A**, A thin section of the glomerulus of a piglet with factor H deficiency. Note the thickened basement membrane and increased numbers of mesangial cells, hence the name *membranoproliferative glomerulonephritis*. **B**, An immunofluorescence photomicrograph of another glomerulus from a factor H-deficient piglet. This is stained with fluorescent anti-C3. The bright fluorescence indicates the presence of C3 deposited in this glomerulus. Compare this figure with [Figure 27-10](#) in [Chapter 27](#). (**A**, Courtesy Johan H. Jansen; **B**, from Jansen JH, Hogasen K, Mollnes TE: *Am J Pathol* 143:1356-1365, 1993.)

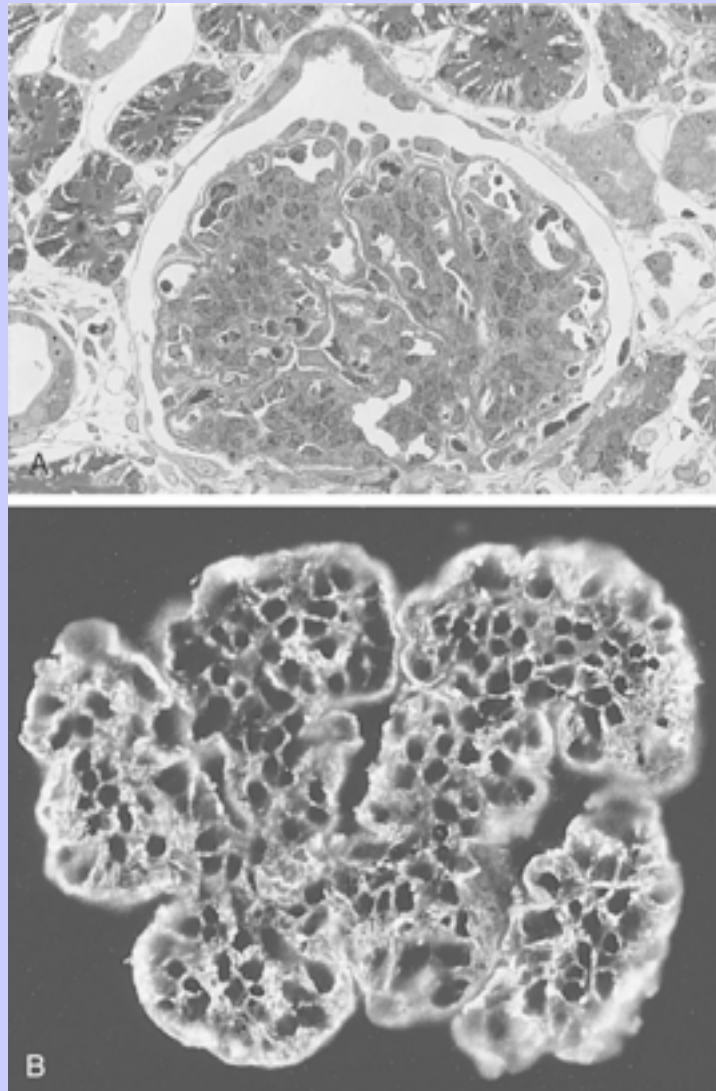
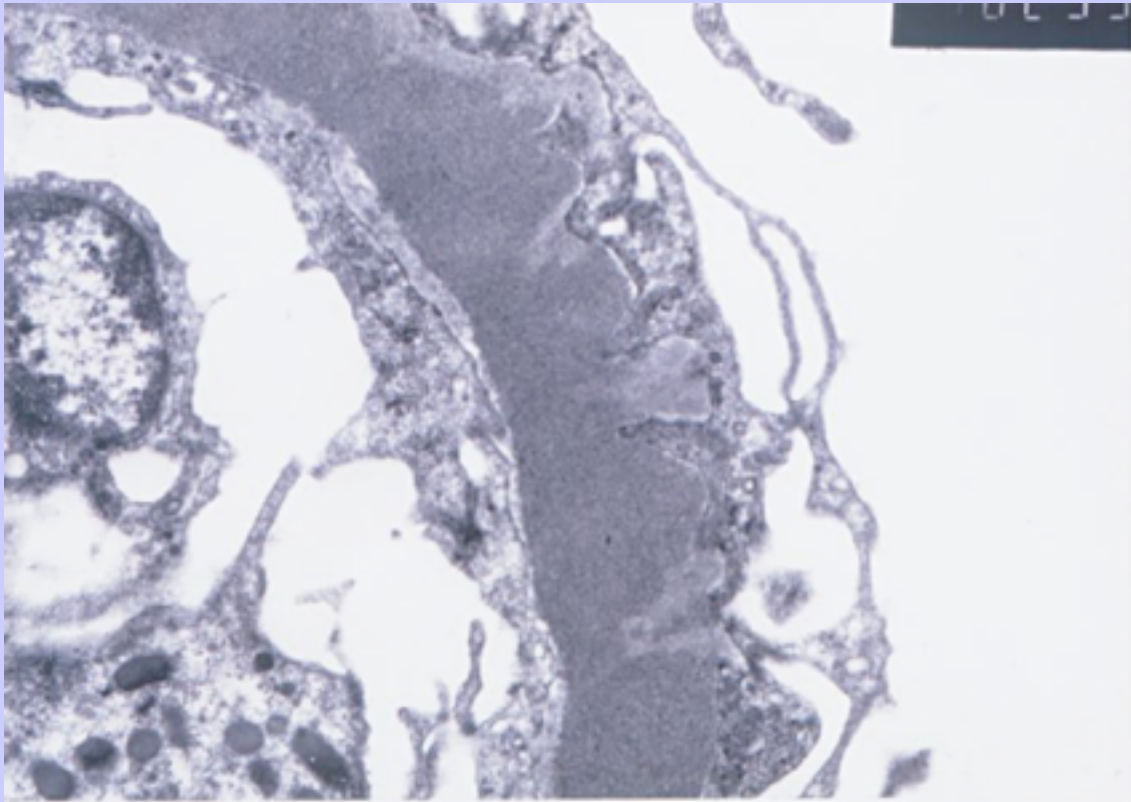


FIGURE 5-18 Electron micrograph showing dense intramembranous deposits in the glomerulus of a piglet with factor H deficiency. (From Jansen JH: APMIS 101:281-289, 1993.)



atrophy of the renal papillae. On light microscopy, alterations are seen in the renal glomeruli, i.e., mesangial cell proliferation and capillary basement membrane thickening ([Figure 5-17](#)). Electron microscopy reveals extensive intramembranous electron-dense deposits within the glomerular basement membranes ([Figure 5-18](#)). This is typical of type II membranoproliferative glomerulonephritis or dense-deposit disease (see [Chapter 27](#)). Indirect immunofluorescence demonstrates massive deposits of C3 but no immunoglobulins in the basement membranes. C3 can be found in the glomeruli before birth, but the morphological changes (mesangial proliferation and intramembranous dense deposits) are never seen before 5 days of age. These pigs have no plasma C3. Nephritic piglets are almost totally deficient in factor H (2% of normal levels), whereas heterozygotes have half the normal levels. If factor H is replaced by plasma transfusions, the progress of the disease can be slowed and piglet survival enhanced. Since heterozygotes can be readily detected by measurement of plasma C3, this disease can be eradicated from affected herds.

5.7.3

Other Complement Deficiencies

MBL deficiency has been described in children, where it results in increased susceptibility to infection. It has not yet been described in domestic animals. In contrast to the severe effects of a C3 deficiency, congenital deficiencies of other complement components in laboratory animals or humans are not necessarily lethal. Thus

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individuals with C6 or C7 deficiencies have been described who are quite healthy. Apparently healthy C6-deficient pigs have been described. The lack of discernible effect of these deficiencies suggests that the terminal portion of the complement pathway leading to lysis may not be biologically essential.

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6 CHAPTER 6 Cell Signaling: Cytokines and Their Receptors

6.1 KEY POINTS

- The immune responses develop as a result of interactions between different cell populations.
- Cells interact by secreting signaling molecules such as cytokines and hormones.
- These signaling molecules bind to specific receptors on target cells.
- When signaling molecules bind to these receptors they cause a cell to alter its behavior through a process called signal transduction. As a result transcription factors are generated. These transcription factors then activate selected genes.
- As a result of gene transcription, cells alter their behavior and secrete new cytokines or other signaling molecules.

The immune system functions through many different cell types sending and receiving messages that are delivered in the form of chemical signals. Molecules secreted by one cell are carried to another, where they bind to receptors on the target cell surface. By receiving signals through their appropriate receptors, the target cells can be stimulated to behave in an appropriate manner. They may be told to divide or stop dividing; they may be stimulated to secrete their own signaling molecules; they may be told to commit suicide. Each cell lives in an environment where it is exposed to many different signaling molecules at any one time. The target cell must integrate these signals in some way and respond appropriately. In this chapter we review the signaling molecules secreted by cells, the receptors that receive these signals, and the way in which the received signals are interpreted by the receiving cell. It should be pointed out that other body systems receive many messages by way of the nervous system. While nerves undoubtedly do connect with cells of the immune system (see [Chapter 17](#)) and regulate some aspects of immunity, this appears to be a relatively unimportant route compared to signaling through soluble mediators.

The cells of the immune system secrete several hundred different proteins that regulate the immune responses by communicating among cells. These proteins are called cytokines ([Box 6-1](#)). Cytokines differ from conventional hormones in several important respects. For example, unlike classical hormones, which tend to affect a single target, cytokines affect many different cell types. Second, immune system cells rarely secrete a single cytokine at a time. For instance, macrophages secrete at least five interleukins (interleukin-1 [IL-1], IL-6, IL-12, IL-18) as well as tumor necrosis factor- α (TNF- α). Third, cytokines are “redundant” in their biological activities in that many different cytokines have similar effects. For example, IL-1, TNF- α , TNF- β , IL-6, high mobility group box protein-1 (HMGB1), and the chemokine CCL3 all act on the brain to cause fever. This complexity has given rise to the concept of a cytokine network, a web of signals transmitted among all the cell types of the immune system mediated by very complex mixtures of cytokines.

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6.2 Box 6-1 Properties of Cytokines

- Short-lived proteins
- Highly diverse structures and receptors
- Can act locally and/or systemically

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- Pleiotropic: affect many different cells
- Redundant: exhibit biologically overlapping functions
- Carefully regulated
- Toxic in high doses

6.3 Box 6-2 Cytokine Nomenclature

The naming of cytokines is the responsibility of the Interleukin Nomenclature Subcommittee of the International Union of Immunological Societies. This naming has been based on the each cytokine's origin and structure, as well as the demonstration of functional effects on leukocytes. Unfortunately, the gene nomenclature committee of the Human Genome Organization has recently assigned interleukin names to molecules based only on sequence similarity to other interleukins. The result has been a rash of duplications and mislabelings. For example, several different proteins have been called interleukin-25 (IL-25). This has caused such confusion that the name IL-25 is no longer in use. The number of "interleukins" has grown rapidly, while their biological significance remains quite unclear.

6.4 CYTOKINE NOMENCLATURE

The nomenclature of the cytokines is not based on any systematic relationship among these proteins. Many were originally named after their cell of origin or the bioassay used to identify them ([Box 6-2](#)).

The interleukins are cytokines that mediate signaling between lymphocytes and other leukocytes. They are numbered sequentially in the order of their discovery. Because their definition is so broad, the interleukins are a heterogeneous mixture of proteins with little in common except their name. As of 2007, 34 different interleukins have been described. As might be expected, we know a lot about some of these molecules while we know very little about others. Likewise some are critical to a successful immune response while others appear to be much less important.

The interferons are cytokines produced in response to virus infection or immune stimulation. Their name is derived from the fact that they interfere with viral RNA and protein synthesis and so block viral replication. There are two major types of interferon. The most important type I interferons are interferon- α (IFN- α) and interferon- β (IFN- β). There is a single type II interferon, called interferon- γ (IFN- γ). Type I interferons are primarily antiviral with a secondary immunoregulatory role. For type II interferons such as IFN- γ , the reverse is the case. Many type I interferons also play an important role in the maintenance of pregnancy.

TNFs are cytokines secreted by macrophages and T cells. As their name suggests, they can kill tumor cells, although this is not their primary function. Thus TNF- α is the key mediator of acute inflammation. The TNFs belong to a family of related cytokines, the TNF superfamily, which is involved in immune regulation and inflammation. Other important members of the TNF superfamily include CD178 (also called CD95L or fas ligand; see [Chapter 16](#)) and CD154 (CD40 ligand).

Many cytokines serve as growth factors (or colony-stimulating factors) and control leukocyte production by regulating stem cell activities. They thereby ensure that the body is supplied with sufficient cells to defend itself.

Chemokines are a family of at least 50 small proteins that play an important role in leukocyte circulation and migration, especially in inflammation. They are chemotactic factors and leukocyte activators. A typical example of a chemokine is CXCL8 (also known as IL-8). Chemokines are described in detail in [Chapter 2](#).

6.5 CYTOKINE FUNCTIONS

Cytokines are produced in response to many different stimuli. The most important of these stimuli are antigens acting through the T cell or B cell antigen receptors, antigen-antibody complexes acting through antibody receptors (*FcRs*), and pathogen-associated molecular patterns (*PAMPs*) such as lipopolysaccharides acting through toll-like receptors (*TLRs*) ([Figure 6-1](#)).

Cytokines act on many different cellular targets. They may, for example, bind to receptors on the cell that produced them and thus have an autocrine effect. Alternatively, they may bind only to receptors on nearby cells (this is called a paracrine effect). Some cytokines may spread throughout the body, affecting target cells in distant locations and thus having an endocrine effect ([Figure 6-2](#)).

When cytokines bind to receptors on target cells, they affect cell behavior. They may induce the target cell to divide or differentiate, or they may stimulate the production of new proteins. Alternatively they may inhibit these effects by preventing division, dif

FIGURE 6-1 Three of the most important reactions that trigger cytokine release are the combination of antigens with their receptors on T and B cells; the combination of pathogen-associated molecular patterns (PAMPs) with toll-like receptors (TLR); and the combination of antibodies with Fc receptors (FcR).

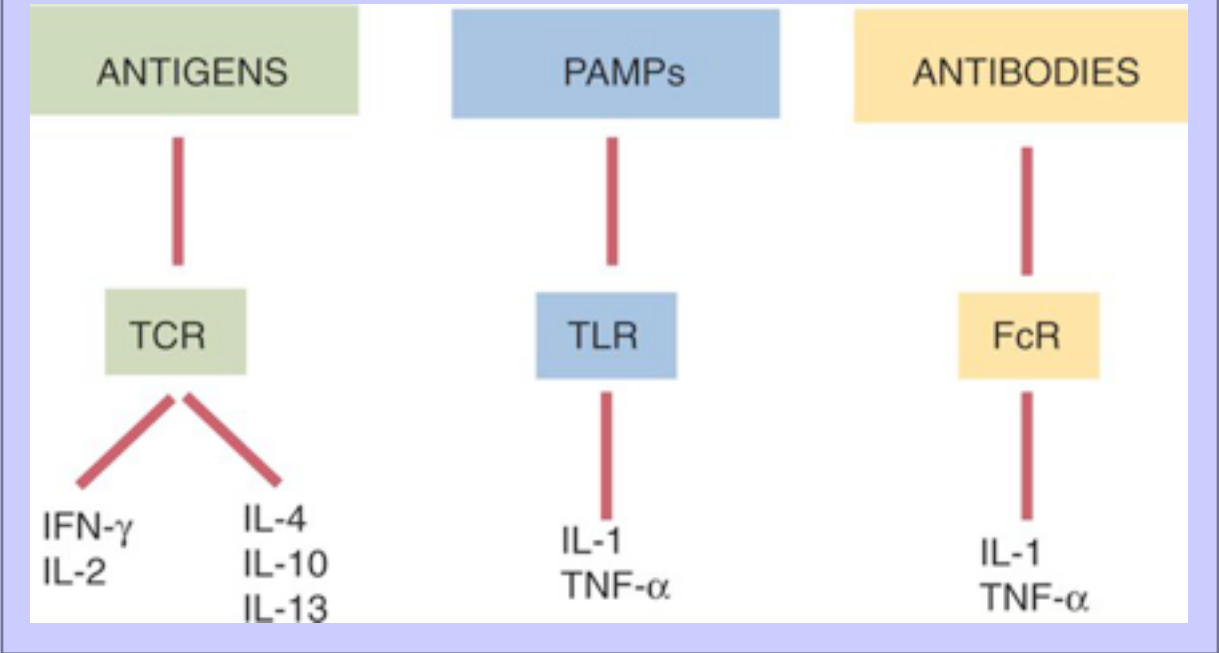
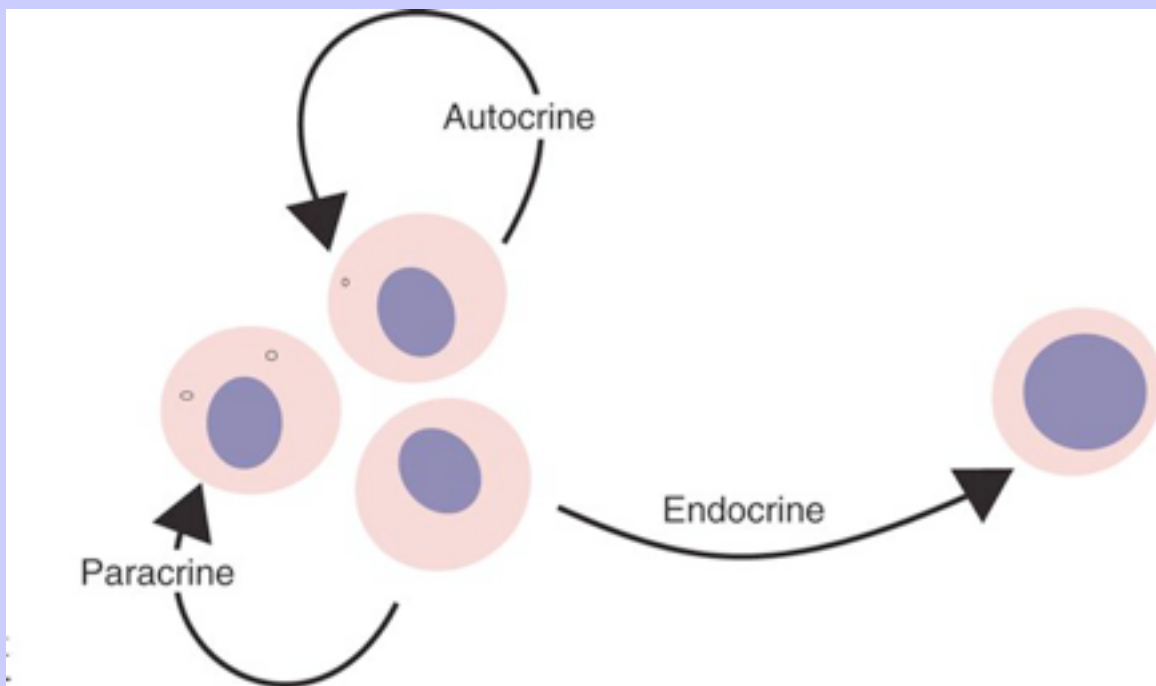


FIGURE 6-2 The distinction among autocrine, paracrine, and endocrine effects. Cytokines differ from hormones in that most of their effects are autocrine or paracrine, whereas hormones act on distant cells in an endocrine fashion.



ferentiation, or new protein synthesis. Most cytokines act on many different target cell types, perhaps inducing different responses in each one, a feature that is called pleiotropy. Conversely, many different cytokines may act on a single target, a feature known as redundancy. For example, IL-3, IL-4, IL-5, and IL-6 all affect B cell function. Some cytokines work best when paired with other cytokines in a process called synergy. For example, the combination of IL-4 and IL-5 stimulates B cells to make immunoglobulin E (IgE) and hence triggers an allergic response. Synergy can also occur in sequence when, for example, one cytokine induces the target cell to express the receptor for another cytokine. Finally, some cytokines have opposing effects and so may antagonize the effects of others. The best example of this is the mutual antagonism of IL-4 and IFN- γ .

Table 6-1 A Molecular Classification of the Cytokines

Structural Families	Structure	Examples
Group 1	Four α helix bundle	IL-2, -3, -4, -5, -6, -7, -9, -11, -13, -15, -21, -23, -30; GM-CSF, erythropoietin, G-CSF, prolactin, leptin
	Interferon subfamily	IFN- α/β , IFN- γ
	Interleukin-10 subfamily	IL-10, -19, -20, -22, -24, -26
Group 2	Beta sheets	TNFs, TGF- β , IL-1 α , IL-1 β , IL-18
Group 3	α Helices and β sheets	Chemokines
Group 4	Mixed motifs	IL-12
Ungrouped	Unique structures	IL-17A-F, IL-14, IL-16

6.6 CYTOKINE STRUCTURE

Cytokines are proteins with diverse structures. They can, however, be classified into several structural families ([Table 6-1](#)).

The largest family, the group 1 cytokines (or hematopoietins), consists of four α -helices bundled together. They include many different interleukins as well as growth hormone and leptin. Within the group 1 cytokines are two major subfamilies of related proteins, the interferon subfamily and the IL-10 subfamily.

In contrast, group 2 cytokines consist of long chain β -sheet structures. They include the TNFs, the IL-1 family, and TGF- β . Group 3 cytokines are small proteins with both α -helices and β -sheets. These include the chemokines and related molecules (see [Chapter 2](#)). Group 4 cytokines are constructed using domains with mixtures of different structural motifs and include IL-12. The IL-17 family, IL-14, and IL-16 are structurally unique proteins and do not belong to any of these major families.

Patterns may also be seen in the biological activities of these cytokines. Thus group 1 cytokines are all involved in immune regulation or stem cell regulation. Group 2 cytokines are mainly involved in the growth and regulation of cells, cell death, and inflammation. Group 3 cytokines are involved in inflammation. The activities of the group 4 cytokines

FIGURE 6-3 The structure of a cytokine receptor. In this case the interleukin-2 (*IL-2*) receptor complex. The complete trimer serves as a high-affinity receptor while the β - γ dimer serves as a low-affinity receptor.

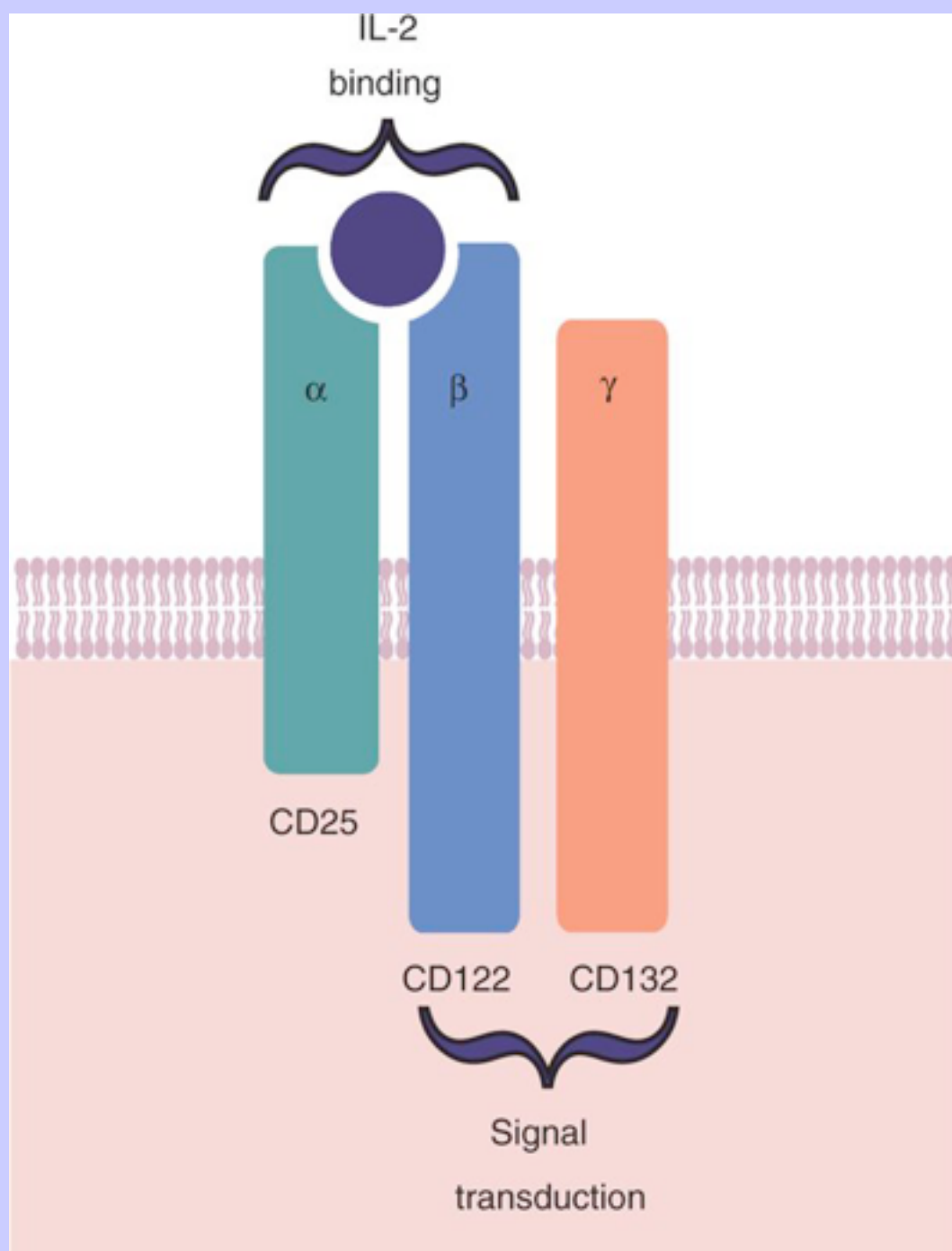
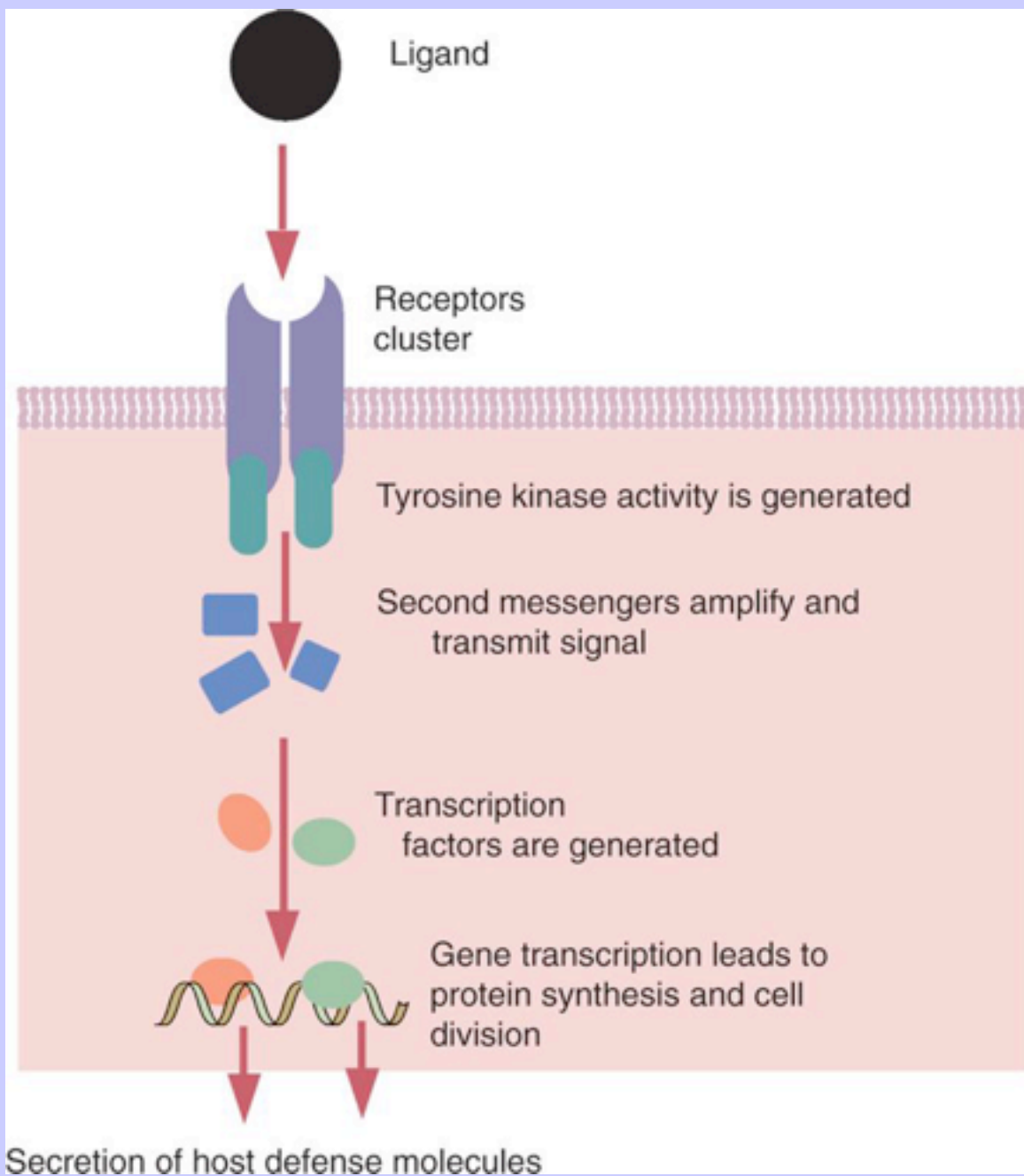


FIGURE 6-4 Generic view of signal transduction involving the activation of tyrosine kinases. Although receptor signaling varies in its details, the overall process of signal transduction has some consistent features shown here.



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depend on their subcomponents. For example, IL-12 is formed by a combination of a group 1 structure with a stem cell receptor, but it acts like a group 1 cytokine.

6.7 CYTOKINE RECEPTORS

Cytokines act through cell surface receptors. These receptors consist of at least two functional units, one for ligand binding and one for signal transduction ([Figure 6-3](#)). These may or may not be on the same protein. Cytokine receptors can also be classified based on their structure.

One class, the channel-linked receptors, serves as transmitter-gated ion channels. Thus the receptor itself is a channel, and binding of its agonist opens that channel allowing ions to pass through it. Channel-linked receptors are found in inflammatory and immune cells, but their roles are unclear. They do not serve as cytokine receptors.

A second class of receptor consists of proteins that also act as tyrosine kinases (TKs) ([Figure 6-4](#)). These are typically growth factor and cytokine receptors. In these cases binding of the ligand to two adjacent receptors forms an active dimer. The receptor site, the membrane-spanning region, and the effector enzyme are usually separate domains of a single protein. Thus when the ligand binds to the extracellular domain and the receptors dimerize, the two TKs are brought together and activate each other. These kinases phosphorylate tyrosine residues on other proteins or even the receptor itself (autophosphorylation). Since many of these other proteins are also TKs, it also converts them to an active state. In this way a cascade of expanding phosphorylations develops within the cell ([Figure 6-5](#)). The phosphorylation triggers changes in cellular activities. Many cytokines and other immunological signals operate through this type of receptor (especially through protein kinases of the Src family). A related class of receptor consists of proteins that are not themselves TKs but can activate TKs associated with the receptors. This type of receptor is also widely employed in the cells of the immune system. Examples of TK-linked receptors include the T cell antigen receptor (TCR) and the B cell antigen receptor. Some of these TKs may transfer their phosphate groups to transcription factors within the nucleus and activate them. Others act indirectly through the production of second messengers.

A large class of receptors is associated with membrane-bound guanosine triphosphate (GTP)-binding

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FIGURE 6-5 The key to cellular activation is the phosphorylation of tyrosine residues by the actions of a tyrosine kinase. For example, phosphorylation of tyrosine by a protein kinase results in phospholipase activation, which leads eventually to cell activation.

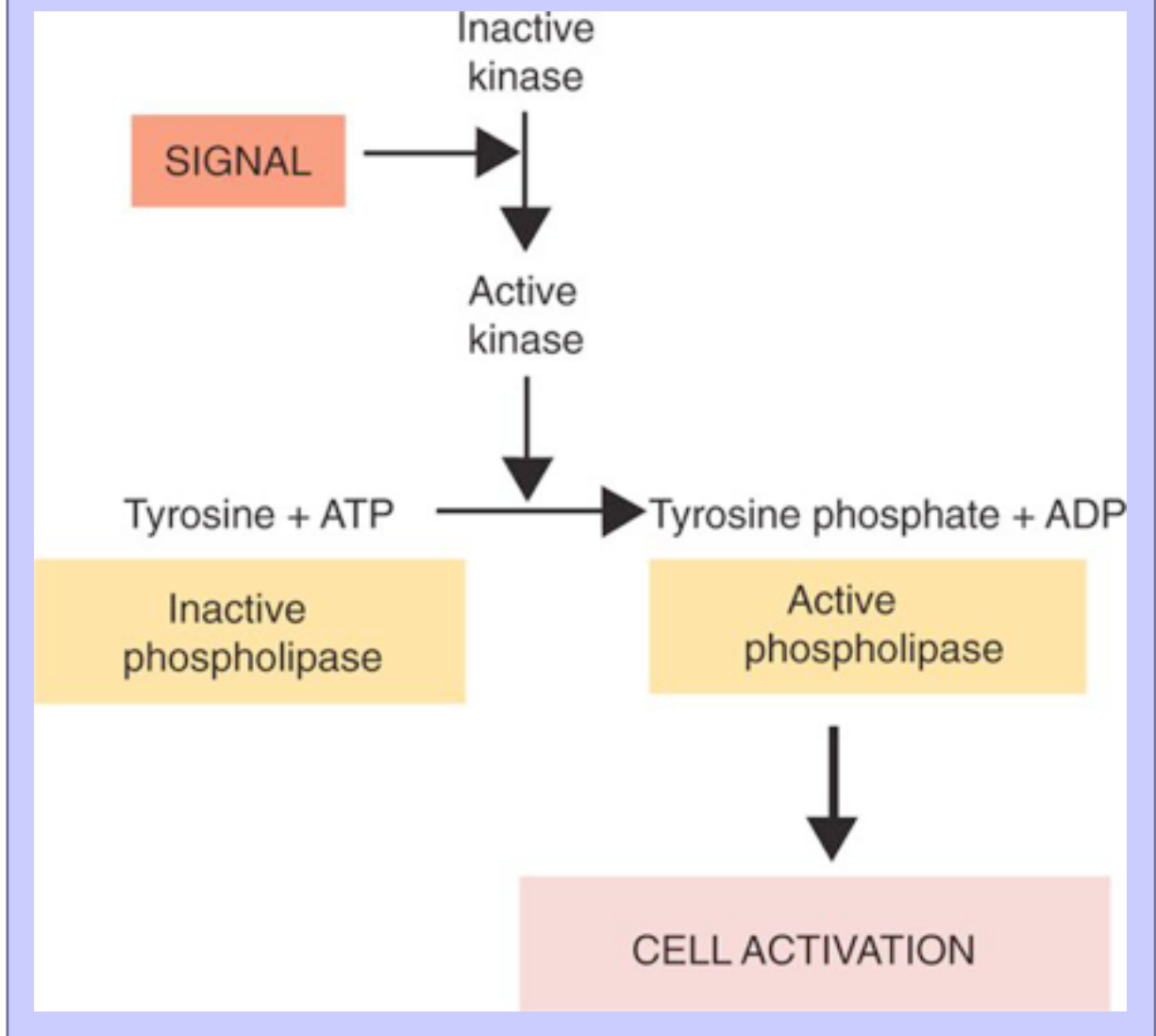
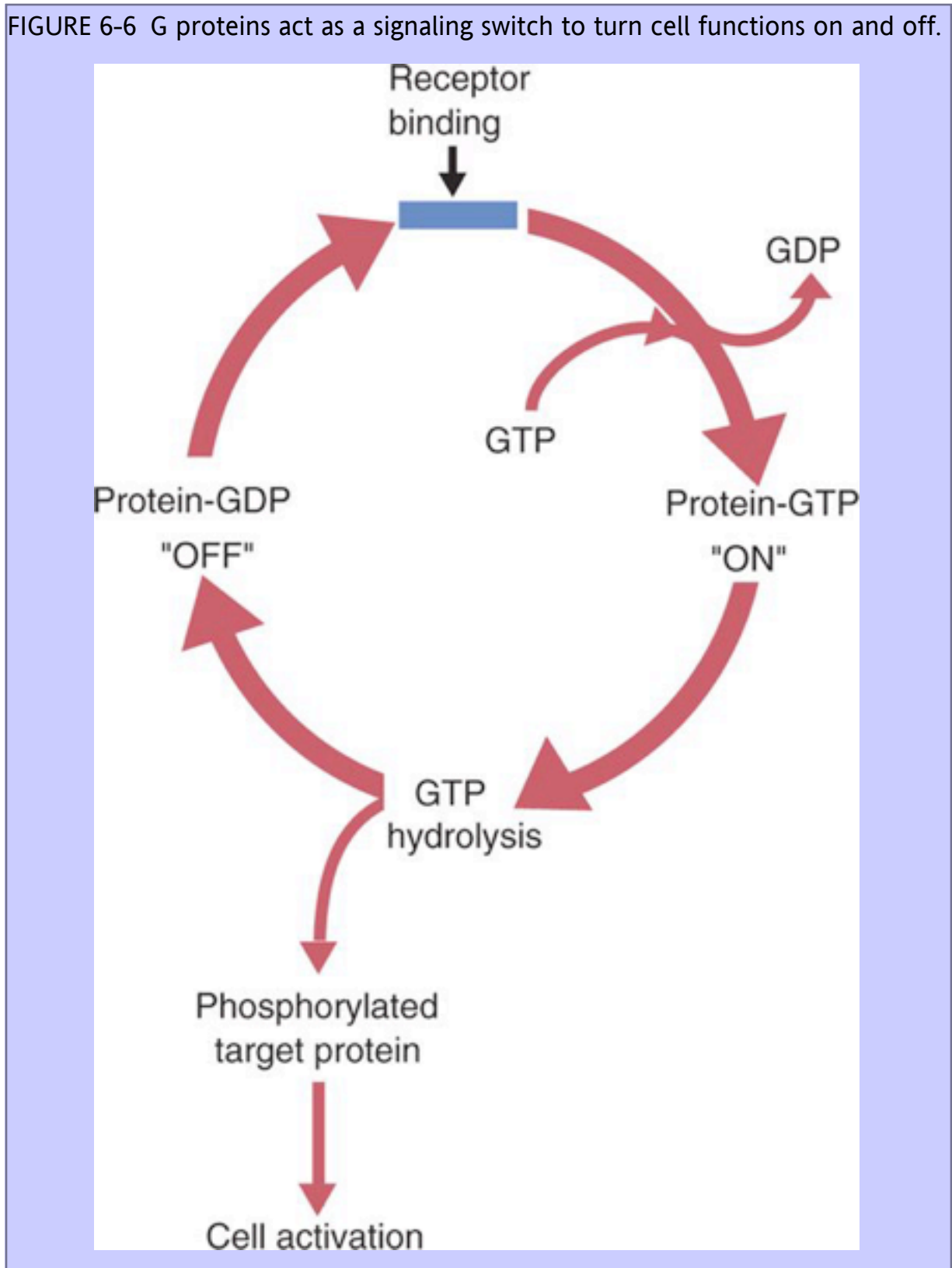


FIGURE 6-6 G proteins act as a signaling switch to turn cell functions on and off.



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proteins, called G proteins. G proteins act as chemical switches and so control many different cellular processes. When inactive, they bind guanosine diphosphate (GDP). When active, they bind GTP. Thus when these receptors bind their ligand, a change in the receptor/G-protein complex results in a loss of GDP and a gain of GTP ([Figure 6-6](#)). The activated G protein then activates other substrates. The GTP is rapidly hydrolyzed to GDP so that the G protein is then turned off. The targets of G proteins can include ion channels, enzymes such as adenylate cyclase, phospholipase C, and some protein kinases. When activated by a G protein, phospholipase C splits the membrane-bound lipid phosphatidylinositol 4,5-bisphosphate (PIP₂) into two messenger molecules, inositol trisphosphate and diacylglycerol ([Figure 6-7](#)). Inositol trisphosphate binds to intracellular receptors releasing Ca²⁺ from internal stores and so increases the concentration of intracellular Ca²⁺. These calcium ions can activate many different proteins. The diacylglycerol remains in the plasma membrane and along with calcium activates an enzyme called protein kinase C. The only immunologic receptors that employ G proteins are the receptors for chemokines—C5a and platelet-activating factor.

A fourth class of receptor activates a neutral sphingomyelinase that then hydrolyzes sphingomyelin in the cell membrane to ceramide. The ceramide then stimulates a ceramide-activated serine-threonine protein kinase that phosphorylates cellular proteins. This mechanism of signal transduction is used by the receptors for IL-1 and IFN- α .

6.7.1

Functional Families

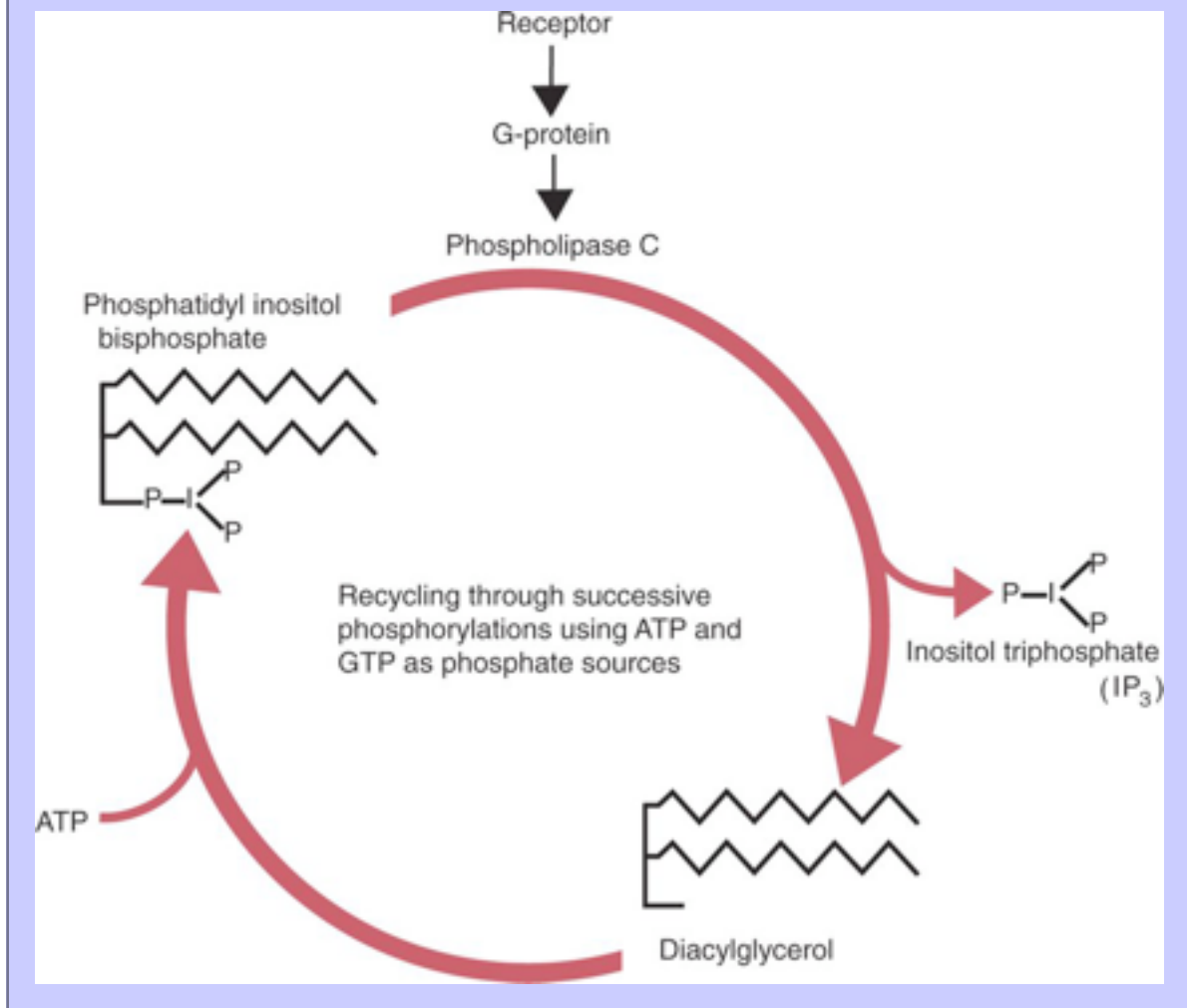
In general, cytokines use receptors that act through TKs. However, within this class, one can identify related families of receptors. For example, the IL-1/TLR receptor family consists of receptors that participate in host responses to injury and infection. They include the receptors for IL-1 and IL-18, as well as the TLRs. The family can be split into the molecules that are IL-1R-like (IL-1R1, IL-18R) and the molecules that are toll-like (TLR). Ligation of these receptors triggers activation of the transcription factor nuclear factor kappa-B (NF- κ B).

Another family, the group I cytokine receptor family, includes the receptors for IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-11, IL-12, IL-13, IL-15, IL-21, granulocyte colony-stimulating factor R (G-CSFR), granulocyte-macrophage colony-stimulating factor R (GM-CSFR), IFN- α / β R, IFN- γ R, and IL-10R ([Figure 6-8](#)). It also includes the common β chain of IL-3, IL-5, and GM-CSF receptors and the common γ chain of IL-2, IL-4, IL-7, IL-9, and IL-15 receptors. These receptor chains dimerize in the presence of the ligand and form complexes with a separate kinase called a Janus kinase (JAK). JAK phosphorylates a cytosolic protein called signal transducer and activator of transcription (STAT). STAT, in turn, dimerizes to form an active transcription factor.

Other group I cytokine receptors bind interferons and cytokines related to IL-10 (IL-19, -20, -22, -24, -26)

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FIGURE 6-7 Activation of cell membrane phospholipase C generates inositol triphosphate and diacylglycerol. These two molecules are messengers that initiate cell activation.



and two IFN- λ molecules (IL-28 and IL-29). These proteins form heterodimers in the presence of the ligand and also signal through the JAK-STAT pathway, leading to cytokine-specific responses. They differ from the class I receptor family in some of their conserved amino acid sequences.

6.8 CYTOKINE REGULATION

Cytokine signaling is regulated in three major ways: by changes in receptor expression, by specific binding proteins, and by cytokines that exert opposite effects. For example, IL-2 receptor expression largely determines the response of T cells to IL-2. T cells express few receptors for IL-2 when resting but many more once activated. In contrast, the activities of IL-1 are regulated by a receptor antagonist called IL-1RA. IL-1RA is an inactive form of IL-1 that binds to the IL-1 receptor but does not stimulate signal transduction. It therefore blocks the activities of active IL-1 ([Figure 6-9](#)). Some cytokines may bind to soluble receptors in body fluids. Examples include the soluble receptors for IL-1,

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IL-2, IL-4, IL-5, IL-6, IL-7, IL-9, TNF- α , and M-CSF. In most cases these soluble receptors compete for cytokine binding with cell surface receptors and hence inhibit their activities. Cytokines such as IL-1, IL-12, and TGF- β may bind to glycosaminoglycans such as heparin or CD44 in connective tissue, where they form a reservoir of readily available molecules. Perhaps the most important way by which cytokine function is regulated is through the opposing effects of different cytokines. For example, IL-4 stimulates B cells to switch to IgE production, whereas IFN- γ suppresses IgE production (see [Chapter 25](#)).

It is also important to bear in mind that at any given time, a single cell is receiving signals from multiple cytokine receptors. It must somehow integrate these multiple signals to produce a coherent response.

6.9 SIGNAL TRANSDUCTION

Cytokines and other molecules act as ligands for their cell surface receptors. Once the ligand binds its receptor, the receptor transmits a signal to the cell to modify its behavior. This conversion of an extracellular signal into a series of intracellular events is called signal transduction. The key components of signal transduction include binding of an agonist to a receptor, activation of a transducer protein by the receptor, secondary activation of other enzymes, generation of new transcription factors, and gene activation leading to altered cell behavior. Because cell signaling must be fast and precise, it is best accomplished by enzyme cascades. Since enzymes can produce or modify a large number

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FIGURE 6-8 The major types of cytokine receptors. Growth factor receptors associate in the presence of their ligand to form a dimer. This brings the tyrosine kinases on the cytoplasmic domains close together. The enzymes are then activated by cross-phosphorylation. Group I cytokine receptors, such as those for interleukin-2 (*IL-2*), associate in the presence of the ligand to form oligomers and form complexes with Janus kinases (*JAK*). When brought together, the JAKs are activated and in turn activate signal transducer and activator of transcription (STAT) proteins. The STAT proteins dissociate and activate transcription factors. Cytokines such as the interferons and IL-10 bind receptors that have a similar mode of action to type I receptors. They differ, however, in their conserved sequences.

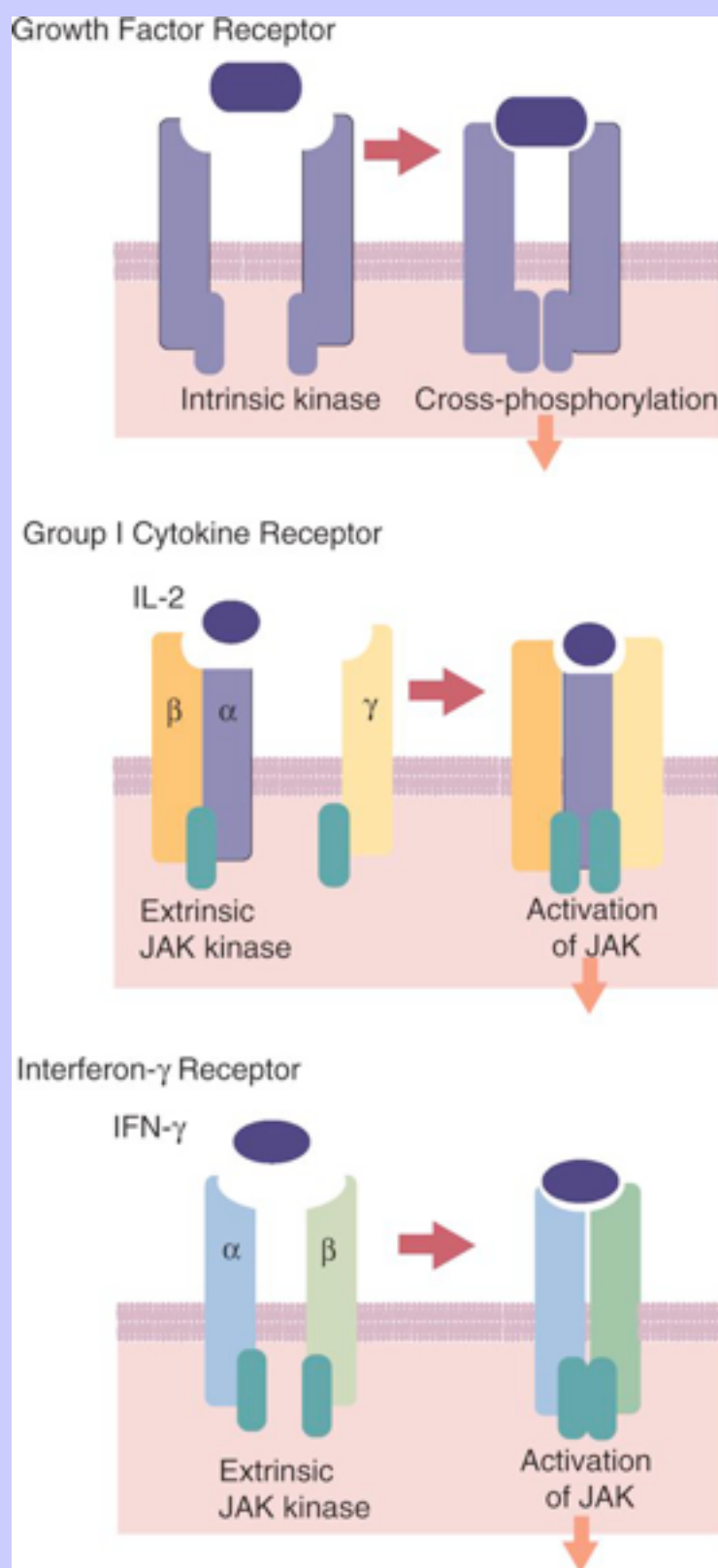
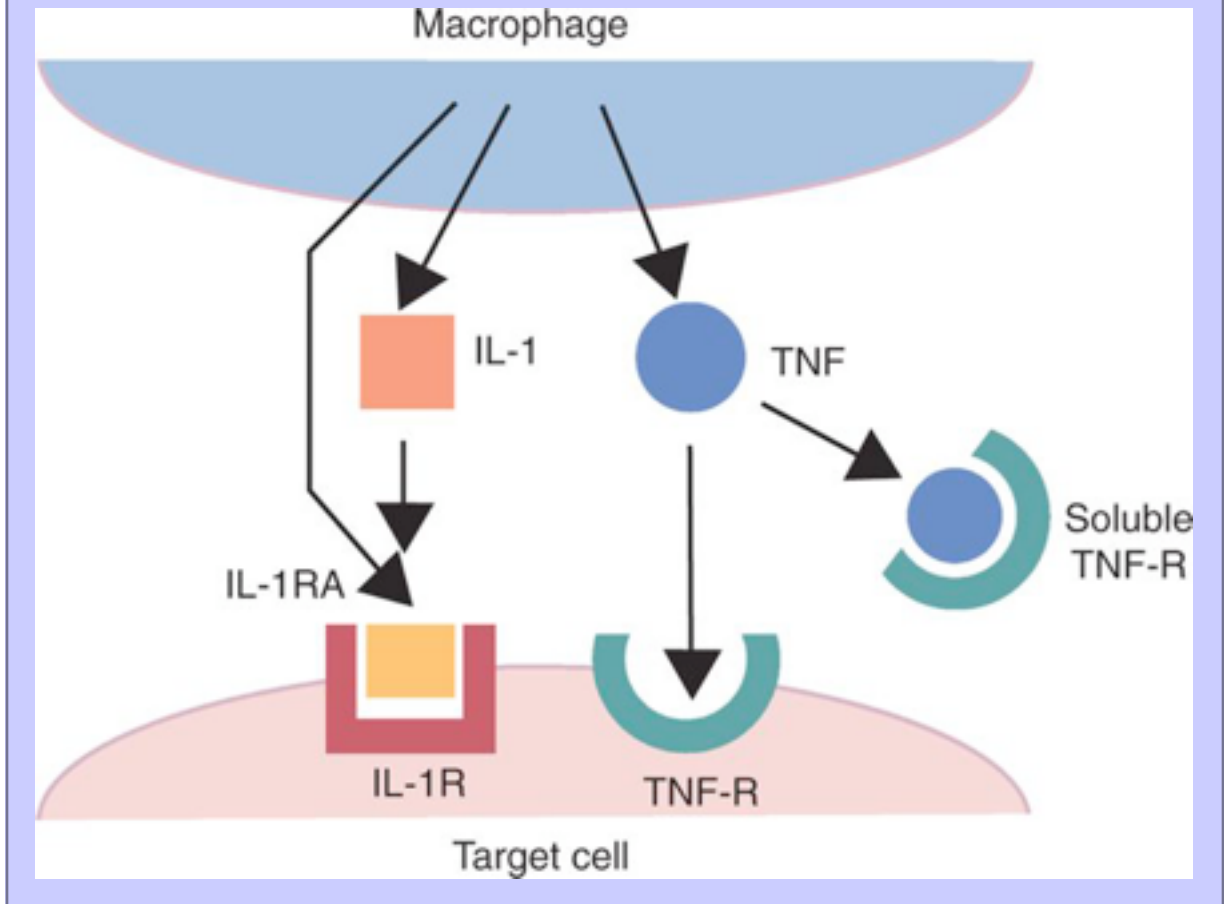
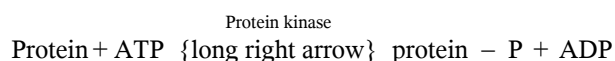


FIGURE 6-9 The control of cytokine activities as exemplified by interleukin-1 (*IL-1*) and tumor necrosis factor- α (TNF- α). *IL-1* activity is regulated by the presence of *IL-1RA*, an inert isoform that binds and blocks the *IL-1R*, preventing signal transduction. Soluble TNF- α R, in contrast, competes for TNF- α with the cell-membrane receptor.



of molecules very rapidly, a pathway that involves the use of several enzymes in sequence can amplify responses very rapidly.

Central to most receptor signaling is the use of protein phosphorylation. Phosphorylation is a form of reversible modification of proteins. All signal transduction systems involve the use of a high-energy compound (e.g., GTP) to modify a protein and send a signal to a cell. Cell growth, cell division, and other critical processes are all regulated by protein phosphorylation. Protein kinases enzymatically phosphorylate the amino acids serine, threonine, and tyrosine.



In some proteins only one amino acid is phosphorylated; in others, multiple amino acids are phosphorylated. Phosphorylated and nonphosphorylated proteins have different functional properties. For example, the

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phosphorylation of serine or threonine activates some enzymes, whereas dephosphorylation has the opposite effect. Phosphorylation of the three key amino acids (serine, threonine, and tyrosine) plays a critical role in the regulation of many cellular functions. When phosphorylated proteins are examined, about 90% of the phosphate is attached to serine and about 10% to threonine. Only about 1/2000 of the phosphate is linked to tyrosine. Thus tyrosine phosphorylation is a rare event despite its being a key mechanism in almost all the signal transduction pathways described in this book.

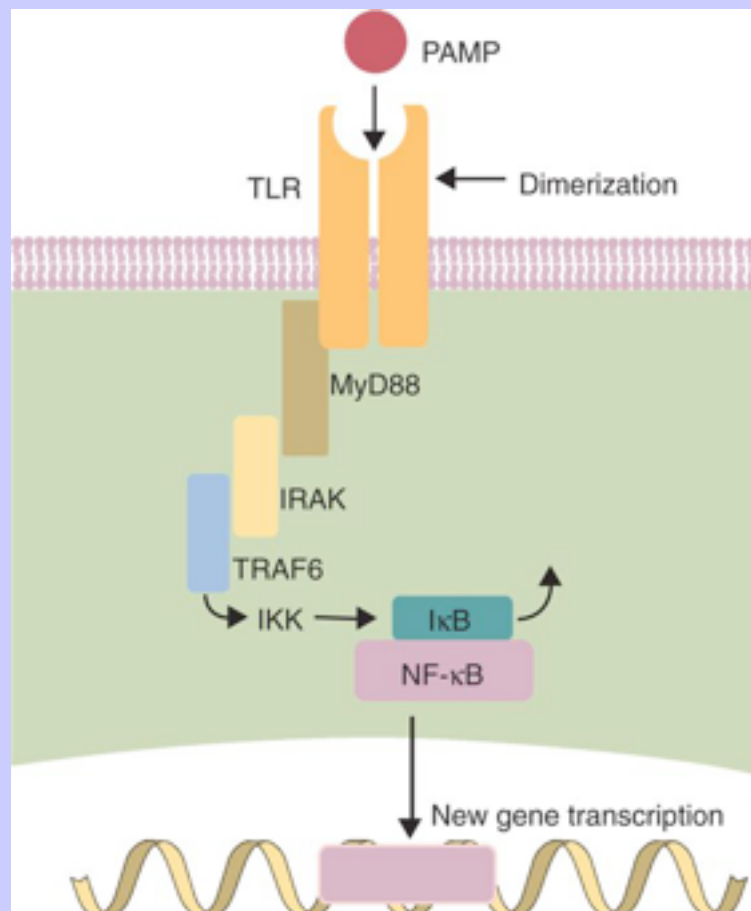
6.9.1 Signal Transduction Pathways

While there are many different pathways of signal transduction, three play key roles in the immune system. These involve the generation of the transcription factors NF- κ B, nuclear factor of activated T cells (NF-AT), and JAK/STAT.

6.9.1.1 The NF- κ B Pathway

The term *NF- κ B* refers to a family of five transcription factors that play a central role in inflammation and

FIGURE 6-10 Signal transduction mediated through toll-like receptors (TLR) generates nuclear factor kappa-B (NF- κ B).



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immunity. All members of the family can form homodimers and heterodimers with one another. NF- κ B exists in the cell cytoplasm in an inactive form through association with the I κ B proteins. The I κ B proteins inhibit NF- κ B activity by masking its nuclear binding site so that in a resting cell, NF- κ B cannot move to the nucleus or activate genes. More than 150 different stimuli have been shown to activate NF- κ B, and more than 150 genes have been shown to be expressed on NF- κ B activation. Three major NF- κ B activation pathways have been identified. The “classical” pathway is involved in proinflammatory signaling. It is activated by inflammatory cytokines (IL-1 and TNF- α), TLRs, and antigen receptors and is essential for innate immunity. The signals induced by these stimuli converge on a central regulator of NF- κ B, the IKK (I κ B kinase) complex. The IKK complex consists of multiple subunits with kinase activity. When a cell is stimulated, the IKK complex phosphorylates I κ B. As a result, I κ B dissociates from the NF- κ B. The newly released I κ B becomes ubiquitinated and is destroyed by proteasomes. This liberates the NF- κ B that can now enter the nucleus, where it binds and activates κ B motif-containing promoters on DNA. This results in activation of many genes including the cytokines IL-1 β , IL-6, IL-18, IL-33, TNF- α , GM-CSF, and IL-4. NF- κ B triggers activation of several different chemokine genes, proangiogenic factors, adhesion molecules such as intercellular adhesion molecule-1, antiapoptotic proteins, inducible enzymes (e.g., inducible nitric oxide synthase, cyclooxygenase-2), and also of more I κ B (which ultimately downregulates NF- κ B activation). Molecules or organisms that block the destruction of I κ B will have antiinflammatory and immunosuppressive effects. Thus corticosteroids stimulate the production of excess I κ B whereas some bacteria can block its degradation. Either way, the activation of cells and the development of inflammation and immune responses will be blocked. A second NF- κ B pathway (sometimes called the alternative pathway) is triggered by a subset of TNF receptors. This pathway is essential for lymphocyte development and activation. The third NF- κ B pathway is activated by DNA-damaging drugs and ultraviolet light. It does not involve IKK activation.

An example of the use of the NF- κ B pathway is seen with TLR responses in macrophages ([Figure 6-10](#)). Occupation of a TLR by a PAMP or by HMGB1 causes the receptor to dimerize and change its shape. As a result, it binds several adaptor molecules of which one, MyD88 (myeloid differentiation primary response gene 88), is the most important. When MyD88 complexes with the TLR, it also binds two kinases (interleukin receptor-associated kinase 1 [IRAK-1] and IRAK-4). IRAK-4 activates IRAK-1, and these in turn recruit tumor necrosis factor receptor-associated factor 6 (TRAF6). TRAF6 and other proteins then activate the IKK complex. Activation of IKK phosphorylates I κ B leading to its destruction and the release of active NF- κ B. The NF- κ B in turn activates genes that encode the cytokines, IL-1, IL-18, and IL-33 as well as the type I interferons. IL-1 activation of NF- κ B shares the same signaling pathways as TLR.

6.9.1.2

The NF-AT Pathway

When an antigen binds to its receptor on a T cell, the receptor (TCR) signals to the T cell. The signal is first transmitted from the antigen-binding TCR to a signal transducing complex called CD3, where it causes the CD3 chains to cluster together in lipid rafts ([Figure 6-11](#)). The CD3 proteins have specific amino acid sequences in their cytoplasmic domains called “immunoreceptor tyrosine-based, activation motifs” (ITAMs). When the chains cluster as an immunological synapse is formed, these ITAMs become binding sites for several TKs. These TKs are members of the Src-kinase family. They include lck and fyn in T cells and NK cells, and lyn and fyn in B cells and mast cells. In T cells, the first TK activated, called lck, phosphorylates the ITAMs. As a result these sites can bind a second TK, called zeta-associated protein-70 (ZAP-70). The bound ZAP-70 is phosphorylated in turn and then can trigger three signaling pathways. Via the second messengers, diacylglycerol and inositol trisphosphate, one pathway leads to the activation of the NF-AT. The inositol trisphosphate also releases calcium ions from intracel

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FIGURE 6-11 Signal transduction mediated through T cell antigen receptors generates three transcription factors: nuclear factor of activated T cells (*NF-AT*), nuclear factor kappa-B (*NF-κB*), and activator protein-1 (*AP-1*). Once T cell antigen receptors cluster they activate several protein kinases. The most important of these is called zeta-associated protein-70. This in turn triggers three signaling pathways and, with appropriate co-stimulation, generates multiple transduction factors. The jun-fos heterodimer (*AP-1*) is required to stimulate the genes for cytokines and their receptors. The final results of the stimulus include cell division or apoptosis as well as cytokine production.

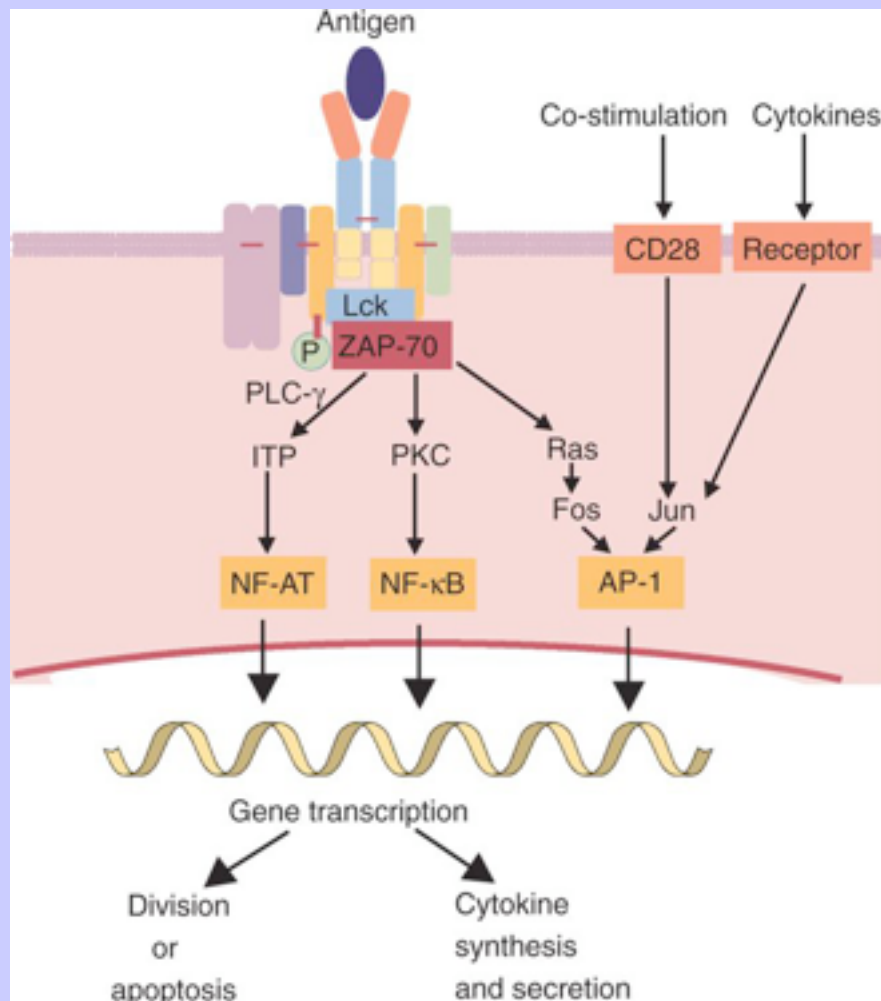
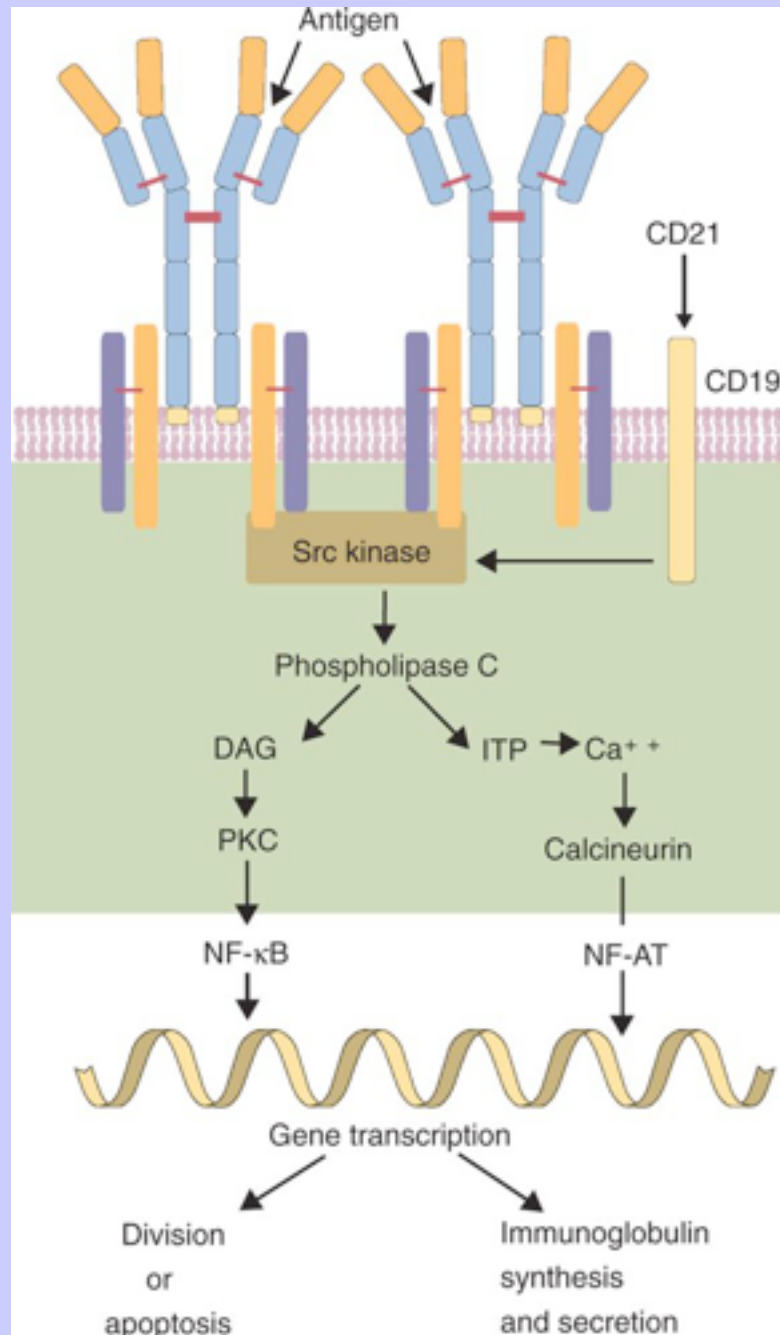


FIGURE 6-12 Signal transduction by two cross-linked B cell receptors activates B cells triggering cell division, differentiation, and immunoglobulin synthesis. Both nuclear factor kappa-B (NF- κ B) and nuclear factor of activated T cells (NF-AT) are involved in B cell signal transduction.



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lular organelles and opens transmembrane channels allowing Ca^{2+} to enter the cell and raising intracellular calcium. This in turn activates a phosphatase called calcineurin, which removes a phosphate from NF-AT. Dephosphorylated NF-AT enters the nucleus and with the help of another transcription factor, activator protein-1 (AP-1), binds to the promoters of at least 100 genes expressed in activated T cells. The potent immunosuppressive drugs tacrolimus and cyclosporine bind to calcineurin and so can block T cell-mediated responses.

In B cells, the adaptor molecules Iga and Igb have ITAMs. When aggregated by antigen and co-stimulated by CD19, the Src kinases lyn and fyn are activated. These in turn activate phospholipase C and eventually generate NF- κ B and NF-AT ([Figure 6-12](#)).

The second pathway triggered by ZAP-70 activates a protein kinase C, which phosphorylates I κ B and so activates NF- κ B. The third pathway activates ras, a GTP-binding protein, that in turn activates fos. At the same time, co-stimulatory signals initiated by another receptor called CD28 lead to the activation of a protein called jun. The fos and jun proteins bind to form AP-1. AP-1 together with NF-AT activates multiple genes. The net effect of these reactions is that T cells enlarge, enter the cell cycle, and synthesize and secrete a mixture of cytokines. These cytokines trigger the next stages of the immune responses.

If the T cell receives other signals, such as those provided by IL-10 or TGF- β , the NF-AT may associate with a different transcription factor called Foxp3. This signal activates a very different set of genes and so converts the cell into a regulatory T cell (T_{reg}) that suppresses immune responses (see [Chapter 17](#)).

6.9.1.3

The JAK-STAT Pathway

The group I cytokine receptors signal through the JAK-STAT signaling pathway. The receptors for the JAK-STAT pathway are two identical single-pass transmembrane proteins. Each of their cytoplasmic ends binds a molecule of a JAK. Almost 40 cytokines use the JAK-STAT pathways including interleukins such as IL-2, IL-7, and IL-11 to IL-13, leptin GM-CSF and IFN- γ . Ligand binding to the receptor causes dimerization that leads to activation of the TK activity of the tightly associated JAK (Janus family TKs). These activated JAK molecules phosphorylate tyrosine residues on one of several STAT proteins (signal transducers and activators of transcription). The phosphorylated STAT proteins then dimerize, dissociate from JAK, and move to the nucleus, where they act as transcription factors and modulate the expression of target genes. There are four JAK and seven STAT family members recognized. A specific JAK-STAT combination is paired with each cytokine receptor. For example, receptors for the growth factors usually use JAK2. Receptors with the common γ chain preferentially use JAK1 and JAK3. The IFN- γ receptor uses JAK 1 and JAK2 (see [Chapter 23](#), [Figure 23-4](#)). The IL-4R uses JAK1 and JAK3. Presumably the outcome of this signaling depends on which combination of JAK and STAT is activated.

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6.10

GENE TRANSCRIPTION

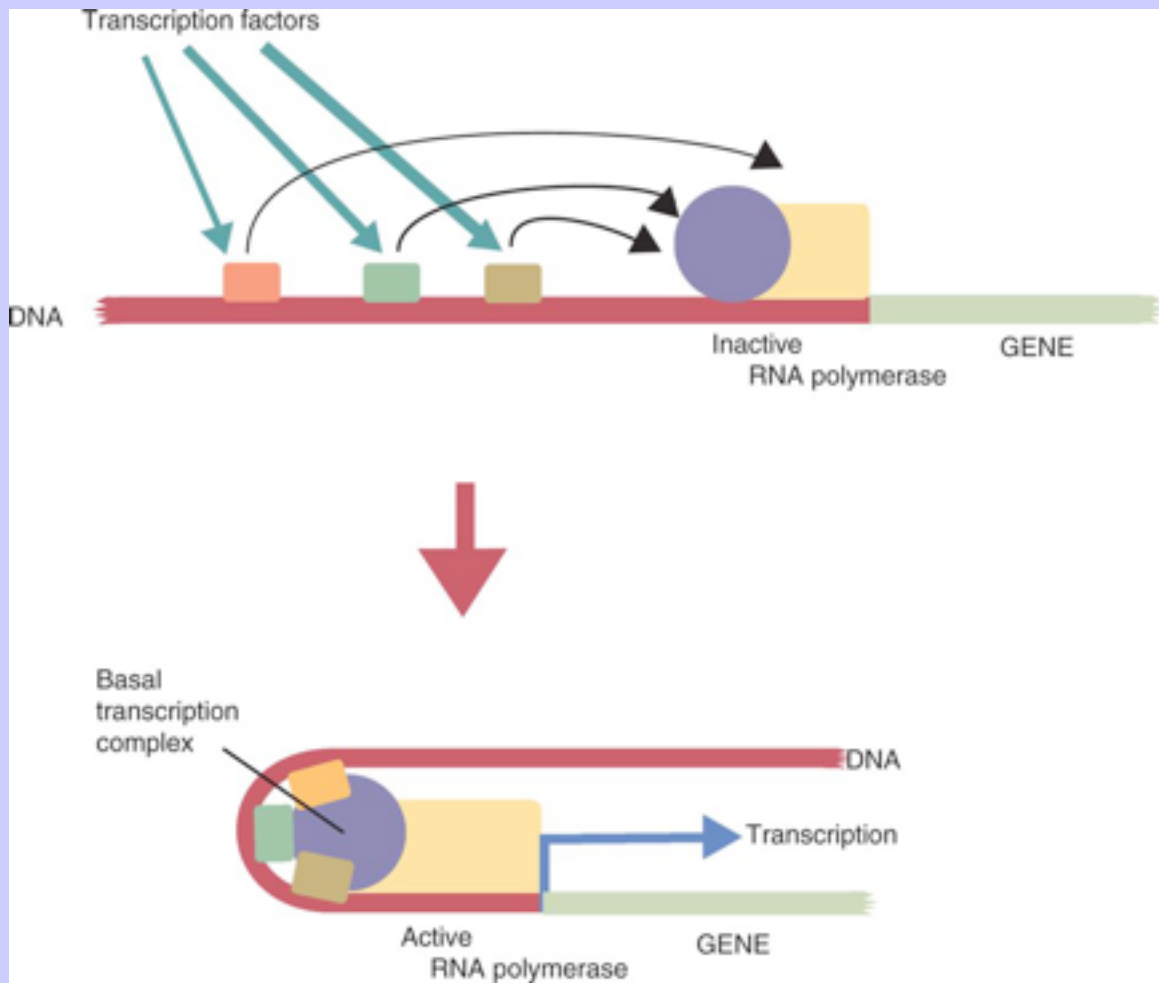
The activity of each gene in a cell is carefully controlled by many different mechanisms. Central to gene control are the transcription factors. Activation of genes depends on the presence of an appropriate mixture of transcription factors. As described above, these transcription factors are only generated when a cell receives an appropriate signal. The transcription factors then collectively activate the appropriate RNA polymerase ([Figure 6-13](#)).

Transcription factors have two binding sites. One site binds DNA; the other is a binding site for other proteins. When a transcription factor is activated, it enters the nucleus and binds to specific DNA control elements located between 50 and 200 bases upstream from the start site of the gene. Transcription factors may also bind to enhancer

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elements located thousands of bases upstream. These bound transcription factors then bind either directly to basal transcription complexes or to a coactivator molecule. This binding leads to assembly of the basal transcription complex. The basal transcription complex together with any

FIGURE 6-13 Transcription factors bind to enhancer elements on DNA located upstream of the genes they activate. Gene transcription is turned on by a carefully regulated RNA polymerase. However, the polymerase can only be turned on when transcription factors activate the basic transcriptional machinery.



coactivator molecules then bind to the RNA polymerase and activate it. In the process it is believed that the conformation of the polymerase changes, activating it and permitting RNA transcription of the selected genes to begin.

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7 CHAPTER 7 Antigens: Triggers of Acquired Immunity

7.1 KEY POINTS

- The acquired immune system is optimized to recognize microbial macromolecules.
- The best antigens are large, complex, stable, foreign proteins.
- Small molecules with a mass of less than 5000 kDa are usually poor antigens.
- Small molecules may be made antigenic by conjugating them to large proteins. Small molecules used as antigens in this way are called haptens.
- The immune system recognizes distinct areas on the surface of foreign antigens. These are called antigenic determinants or epitopes.

Until now we have considered only the body's innate reactions to microbial invasion. Innate responses are triggered by recognition of pathogen-associated molecular patterns (PAMPs) such as CpG DNA and lipopolysaccharide. The triggering of inflammation and the mobilization of phagocytic cells such as neutrophils and macrophages induced by these molecules contribute to the rapid destruction of microbial invaders. Although this may be sufficient to protect the body, it cannot always provide the level of immunity required to ensure resistance to infection. Thus a more potent immune response should recognize all the foreign molecules on an invading microbe. In addition, an ideal defensive response would be able to learn from this experience and, given time, develop more efficient procedures to combat subsequent invasions. This adaptive response is the function of the acquired immune system.

During an acquired immune response, molecules from invading organisms are captured, processed, and presented to the cells of the immune system. When appropriately presented, these antigens will trigger a powerful protective immune response that ensures an animal's survival. In addition, the immune system "remembers" these antigens and responds even more effectively if it encounters them subsequently.

7.2 ANTIGENS

Since the function of the acquired immune system is to defend the body against invading microorganisms, it is essential that these organisms be recognized as soon as they invade the body. The body must be able to recognize that these are foreign (and dangerous) if they are to stimulate an immune response. The innate immune system recognizes a limited number of PAMPs that are characteristic of major groups of pathogens. The acquired immune system, however, can recognize and respond to a vast array of foreign molecular structures. These molecular structures are called antigens.

7.3 MICROBIAL ANTIGENS

7.3.1 Bacterial Antigens

Bacteria are ovoid or spherical organisms consisting of a cytoplasm containing the essential elements of cell structure surrounded by a lipid-rich cytoplasmic membrane ([Figure 7-1](#)). Outside the cytoplasmic membrane is a thick, carbohydrate-rich cell wall. The major components of the bacterial surface thus include the cell wall and its

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associated protein structures—the capsule, the pili, and the flagella. The cell wall of Gram-positive organisms is largely composed of peptidoglycan (chains of alternating *N*-acetyl glucosamine and *N*-acetyl muramic acid cross-linked by short peptide side chains; see [Chapter 2, Figure 2-2](#)). Gram-positive cell walls also contain lipoteichoic acids that are involved in transport of ions across the cell wall. The cell wall in Gram-negative organisms consists of a thin layer of peptidoglycan covered by an outer membrane consisting of a lipopolysaccharide. Most of the antigenicity of Gram-negative bacteria is associated with this component. It consists of an oligosaccharide attached to a lipid (lipid A) and to a series of repeating trisaccharides. The structure of these trisaccharides determines the antigenicity of the organism. Many bacteria are classified according to this antigenic structure. For example, the genus *Salmonella* contains a major species, *Salmonella enteritica*, that is then divided into more than 2300 serovars based on antigenicity. These polysaccharide antigens are called O antigens. When an animal is infected, the outer cell wall lipopolysaccharides of Gram-negative bacteria bind to toll-like and other pattern-recognition receptors and so induce the production of a mixture of cytokines. Because these cytokines are toxic, bacterial lipopolysaccharides are also called endotoxins.

Bacterial capsules consist of polysaccharides (except in *Bacillus anthracis*, where the capsule consists of proteins). These polysaccharides are usually good antigens. Capsules protect bacteria against phagocytosis (see [Chapter 3](#)), and anticapsular antibodies can protect an infected animal. Capsular antigens are collectively called K antigens.

Pili and fimbriae are short projections that cover the surfaces of some Gram-negative bacteria; they are classified as F or K antigens. Pili attach the bacteria to other bacteria and play a role in bacterial conjugation. Fimbriae attach bacteria to surfaces. Antibodies to fimbriae may have an important protective function since they can prevent bacteria from sticking to body surfaces. Bacterial flagella consist of a single protein called flagellin. Flagellar antigens are collectively called H antigens.

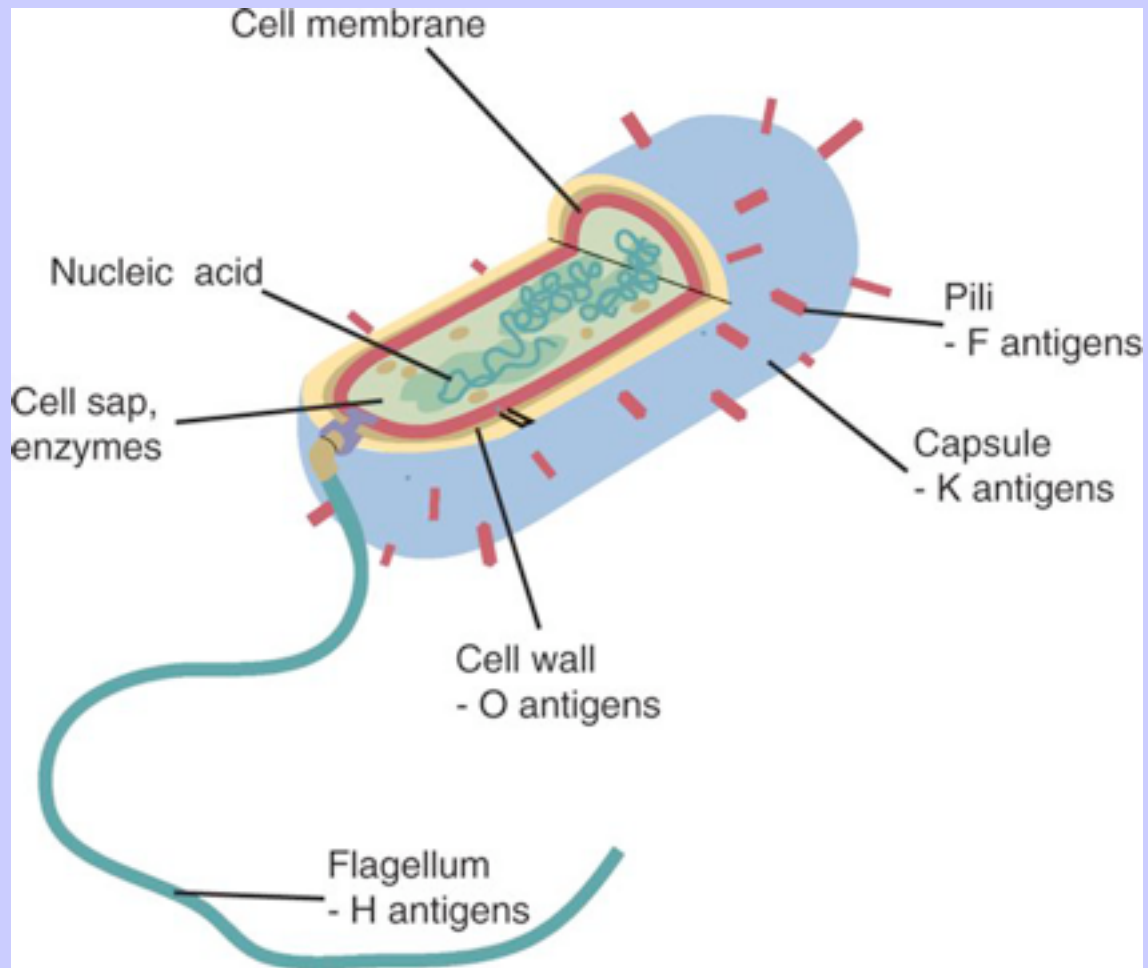
Other significant bacterial antigens include the porins, the heat-shock proteins, and the exotoxins. The porins are proteins that form the pores on the surface of Gram-negative organisms. Heat-shock proteins are generated in large numbers in stressed bacteria. The exotoxins are toxic proteins secreted by bacteria or released into the surrounding environment when they die. Exotoxins are highly immunogenic proteins that, when an animal is infected, stimulate the production of antibodies called antitoxins. Many exotoxins, when treated with a mild protein-denaturing agent such as formaldehyde, lose their toxicity but retain their antigenicity. Toxins modified in this way are called toxoids. Toxoids may be used to prevent disease caused by toxigenic bacteria such as *Clostridium tetani*. Bacterial nucleic acids rich in unmethylated CpG sequences serve both as effective antigens for the acquired immune system and as potent stimulators of innate immunity acting through toll-like receptors.

7.3.2

Viral Antigens

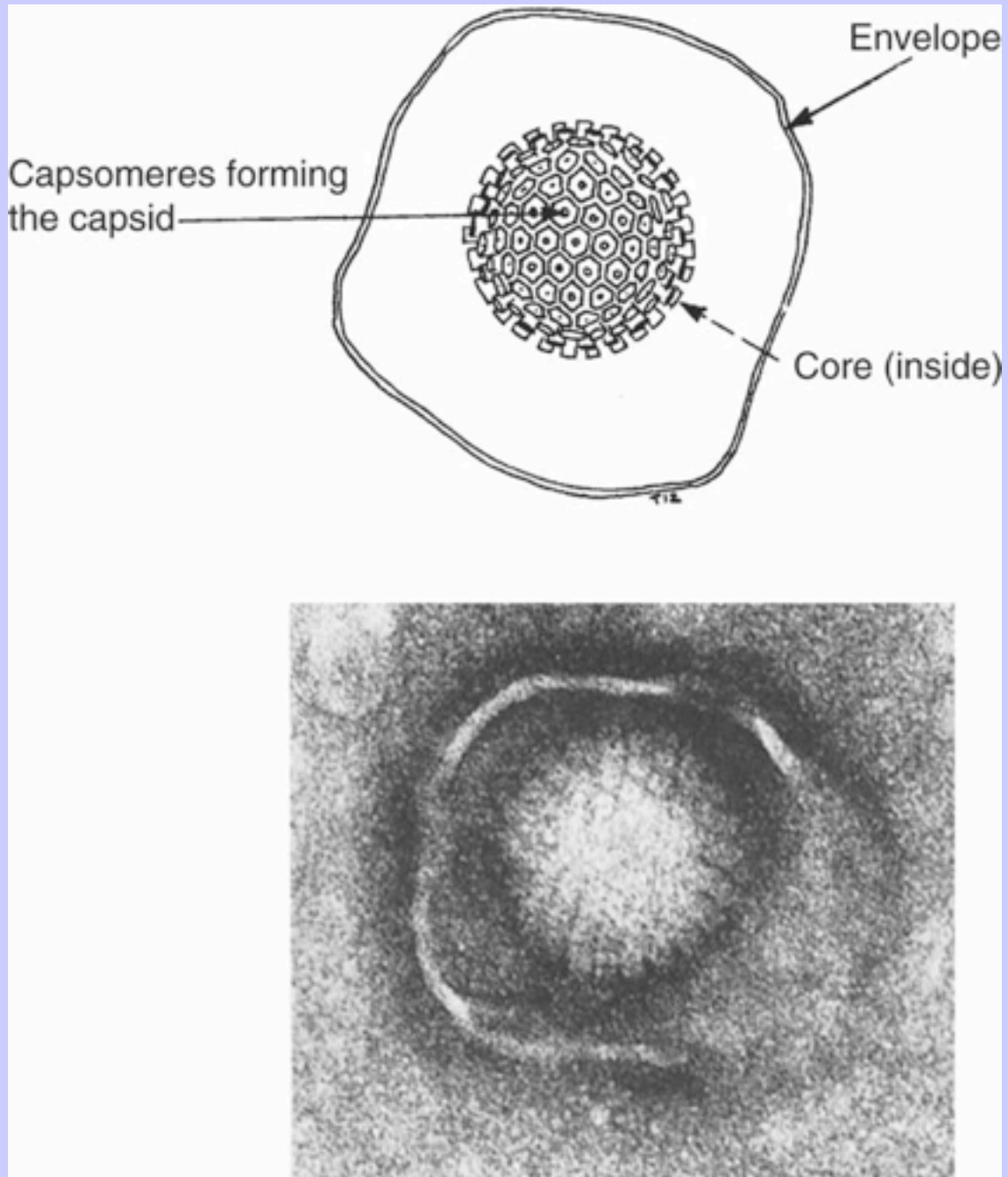
Viruses are very small organisms that can grow only inside living cells. They are thus “obligate,” intracellular parasites. Viruses have a relatively simple structure consisting of a nucleic acid core surrounded by a protein layer ([Figure 7-2](#)). This protein layer is termed the capsid, and it consists of multiple subunits called capsomeres. The capsid proteins are good antigens, highly capable of stimulating antibody formation. Some viruses may also be surrounded by an envelope containing lipoproteins and glycoproteins. A complete viral particle is called a virion. When a virus infects an animal, the proteins in the virions act as antigens

FIGURE 7-1 The structure of a typical bacterium and the location of its most important antigens.



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FIGURE 7-2 The structure of a virus. This is an electron micrograph of equine herpesvirus type 4 magnified 184,000 times. The virus is negatively stained—that is, the electron-dense “dye” has filled the low areas on the virion, leaving the higher areas unstained. (Courtesy Dr. J. Thorsen.)



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and trigger an acquired immune response. Viruses, however, are not always found free in the circulation but live within cells, where they are protected from the unwelcome attentions of antibodies. Indeed, viral nucleic acid can be integrated into a cell's genome. In this situation, the viral genes code for new proteins, some of which are carried to the surface of infected cells. These proteins, although they are synthesized within an animal's cells, are still considered foreign and can provoke acquired immunity. These newly synthesized foreign proteins are called endogenous antigens to distinguish them from the foreign antigens that enter from the outside and are called exogenous antigens.

7.3.3

Other Microbial Antigens

In addition to bacteria and viruses, animals may be invaded by fungi, protozoan parasites, and even by parasitic worms (helminths). Each of these organisms consists of many different structures composed of proteins, carbohydrates, lipids, and nucleic acids. Some of these can serve as antigens and trigger acquired immunity. However, their antigenicity does vary, and the immune responses triggered by these organisms do not always protect an animal or eliminate the invader.

7.4

NONMICROBIAL ANTIGENS

Invading microorganisms are not the only source of foreign material entering the body. Food may contain foreign molecules that under some circumstances trigger immune responses and cause an allergic reaction. Likewise, inhaled dusts can contain antigenic particles such as fungal spores or pollen grains, and antigens from these may enter the body through the respiratory system. Foreign molecules may be injected directly into the body through a snake or mosquito bite, or they may be injected deliberately by a veterinarian administering a vaccine or a blood transfusion. Furthermore, foreign proteins may be injected into animals for experimental purposes. Organ grafts are an effective way of administering a large amount of foreign material to an animal.

7.4.1

Cell Surface Antigens

The cytoplasmic membrane of every mammalian cell consists of a mosaic of protein molecules immersed in a fluid lipid bilayer. Most of these proteins can act as antigens if they are injected into an animal of another species or even into a different animal of the same species. For example, glycoproteins known as blood group antigens are found on the surface of red blood cells. Early attempts to transfuse blood between unrelated individuals usually met with disaster because the transfused cells were rapidly destroyed even though the recipient had never before received a transfusion. Investigation revealed that the problem was due to the presence of naturally occurring antibodies against these red cell glycoproteins (see [Chapter 26](#)).

Nucleated cells, such as leukocytes, possess hundreds of different protein molecules on their cytoplasmic membrane. These proteins are good antigens and readily provoke an immune response when injected experimentally into an animal of a different species. These surface molecules are classified by the cluster of differentiation system (see [Chapter 2](#)). Other cell surface proteins may provoke an immune response (such as graft rejection) if transferred into a genetically different individual of the same species. The most important cell surface proteins that trigger graft rejection are called major histocompatibility complex molecules. These molecules are of such importance in immunology that they warrant a complete chapter of their own (see [Chapter 9](#)).

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7.4.2 Autoantigens

In some situations (and not always abnormal ones), an immune response may be directed against normal body components. This is called an autoimmune response. Antigens that induce this autoimmunity are called autoantigens. They can include hormones such as thyroglobulin; structural components such as basement membranes; complex lipids such as myelin; intracellular components, such as the mitochondrial proteins, nucleic acids, or nucleoproteins; and cell surface proteins, such as hormone receptors. The production of these autoantibodies and the consequences of this production are discussed in detail in [Chapter 31](#).

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7.5 WHAT MAKES A GOOD ANTIGEN?

Molecules vary in their ability to act as antigens (their antigenicity) and stimulate an immune response ([Figure 7-3](#)). In general, foreign proteins make the best antigens, especially if they are big. (A molecular weight greater than 1 kDa is best.) Many of the major antigens of microorganisms such as the clostridial toxins, bacterial flagella, virus capsids, and protozoan cell membranes are proteins. Other important antigenic proteins include components of snake venoms, serum proteins, cell surface proteins, milk and food proteins, hormones, and even antibody molecules themselves.

Simple polysaccharides, such as starch or glycogen, are not good antigens simply because they are degraded before the immune system has had time to respond to them. More complex carbohydrates, however, may be of immunological importance, especially if bound to proteins. These include the major cell wall antigens of Gram-negative bacteria and the blood group antigens of red blood cells. Many of the so-called natural antibodies found in the serum of unimmunized animals are directed against polysaccharides and probably arise as a result of exposure to glycoproteins or carbohydrates from the normal intestinal flora or from food. To this extent they can also be considered to be part of the innate immune system.

Lipids tend to be poor antigens because of their wide distribution, relative simplicity, structural instability, and rapid metabolism. Nevertheless, when linked to proteins or polysaccharides, lipids may be able to trigger immune responses.

Mammalian nucleic acids are very poor antigens because of their relative simplicity and flexibility and because they are very rapidly degraded. Microbial nucleic acids, on the other hand, have a structure very different from that found in eukaryotes with many unmethylated CpG sequences. As a result, they can stimulate a potent acquired immune response. It is perhaps for this reason that autoantibodies to nucleic acids are a characteristic feature of several important autoimmune diseases (see [Chapter 33](#)).

Proteins are the most effective antigens because they have properties that best trigger an immune response. (More correctly, the acquired immune system is optimized to trap, process, and then recognize foreign proteins.) Thus large molecules are better antigens than small molecules, and proteins can be very large indeed ([Figure 7-4](#)). For example, hemocya

FIGURE 7-3 The factors that significantly influence the antigenicity of a molecule. Of these, either excessive or insufficient stability will reduce antigenicity. The best antigens are large, complex, and foreign. However, their ability to stimulate an immune response is also determined by their route of administration, by the amount of antigen administered, and by the genetic makeup of the immunized animal.

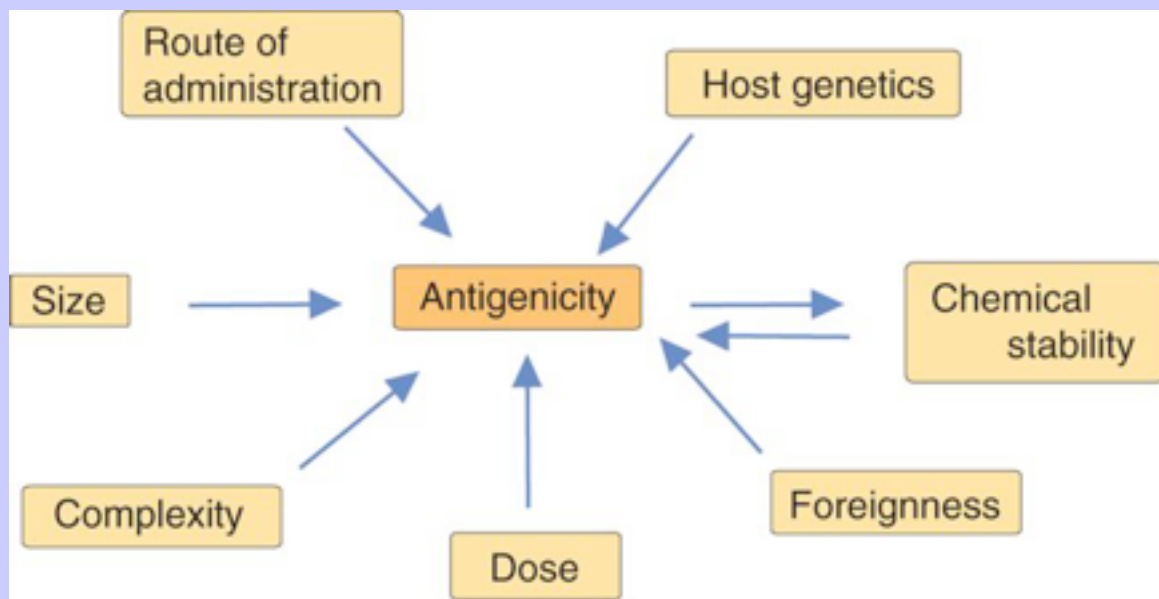
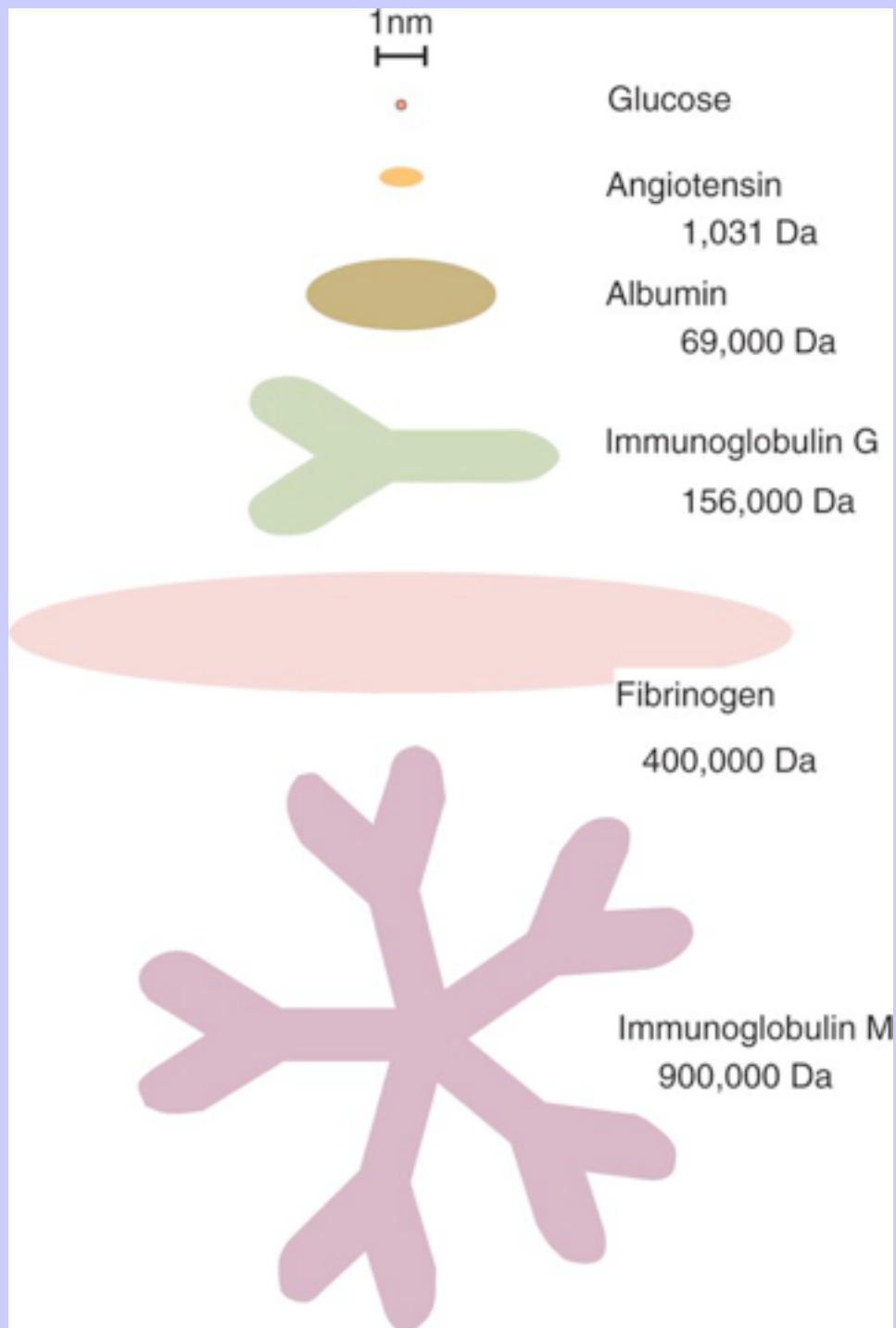


FIGURE 7-4 The relative sizes of several significant antigens. Size does matter—big molecules are generally much more antigenic than small molecules. Molecules as small as angiotensin are poor antigens.



nin, a very large protein from invertebrate blood (670 kDa) is a potent antigen. Serum albumin from other mammals (69 kDa) is a fairly good antigen but may also provoke tolerance. The small peptide hormone angiotensin (1031 Da) is a poor antigen. Similarly, the more complex an antigen is, the better. For example, starch and other simple repeating polymers are poor antigens, but complex bacterial lipopolysaccharides are good. Complex proteins containing many different amino acids, especially aromatic ones, are better antigens than large, repeating polymers, such as the lipids, carbohydrates, and nucleic acids.

Structural stability is an important feature of good antigens, especially those that trigger antibody responses. To bind to a foreign molecule, the cell surface receptors of the immune system must recognize its shape. Consequently, highly flexible molecules that have no fixed shape are poor antigens. For example, gelatin, a protein well known for its structural instability (which is why it can wobble), is a poor antigen unless it is stabilized by the incorporation of tyrosine or tryptophan molecules, which cross-link the peptide chains. Similarly, flagellin, the major protein of bacterial flagella, is a structurally unstable, weak antigen. Its stability, and thus its antigenicity, is greatly enhanced by polymerization. Remember too that the route of antigen administration, its dose, and the genetics of the recipient animal also influence antigenicity.

Clearly not all foreign molecules can stimulate an immune response. Stainless steel bone pins and plastic heart valves, for example, are commonly implanted in humans without triggering an immune response. The lack of antigenicity in the large organic polymers, such as the plastics, is due not only to their molecular uniformity but also to their inertness. These polymers cannot be degraded and processed by cells to a form suitable for triggering an immune response. Conversely, since immune responses are antigen-driven, foreign molecules that are unstable and destroyed very rapidly may not persist for a sufficient time to stimulate an immune response.

7.5.1

Foreignness

The cells that respond to antigens (antigen-sensitive cells) are selected so that their receptors do not normally bind to molecules originating within an animal (self-antigens). They will bind and respond, however, to foreign molecules that differ even in minor respects from those normally found within the body. This lack of reactivity of the acquired immune system to normal body components occurs because cells whose receptors bind self-antigens are selectively killed. During their development, the cells are exposed to self-antigens, and those cells that respond are killed in a process called negative selection.

The immunogenicity of a molecule also depends on its degree of foreignness. The greater the difference in molecular structure between a foreign antigen and an animal's own antigens, the greater will be the intensity of the immune response. For example, a kidney graft from an identical twin will be readily accepted because its proteins are identical to those on the recipient's own kidney. A kidney graft from an unrelated animal of the same species will be rejected in about 10 days unless drugs are used to control the rejection. A kidney graft between different species such as one from a pig to a dog will be rejected within a few hours despite the use of immunosuppressive drugs.

7.6

EPITOPES

Foreign particles, such as bacteria, nucleated cells, and red blood cells, are a complex mixture of proteins, glycoproteins, polysaccharides, lipopolysaccharides, lipids, and nucleoproteins. The acquired immune response against such a foreign particle is therefore a mixture of many simultaneous immune responses against each of the foreign molecules in the mixture.

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A single large molecule such as a protein can also be shown to stimulate multiple immune responses. Large molecules have specific regions, against which immune responses are directed. These regions, usually on the surface of the molecule, are called epitopes, or antigenic determinants ([Figure 7-5](#)). In a large, complex protein molecule, many different epitopes may be recognized by the immune system, but some are much more immunogenic than others. Thus animals may respond to a few favored epitopes, and the remainder of the molecule may be virtually nonimmunogenic. Such epitopes are said to be immunodominant. In general, the number of epitopes on a molecule is directly related to its size: there is usually about 1 epitope for each 5 kDa of a protein. When we describe a molecule as “foreign,” therefore, we are implying that it contains epitopes that are not found on self-antigens. The cells of the immune system recognize and respond to foreign epitopes. A good example of a well-defined epitope is the peptide “proline-glutamic acid-proline-lysine” that binds to antibodies against the bacterium *Streptococcus equi*. Presumably the shape of this peptide is identical to the major antigenic determinant on *S. equi*.

7.6.1

Haptens

A small molecule such as a drug or a hormone with a molecular weight of less than 1 kDa is far too small to be appropriately processed and presented to the immune system. As a result, it is not immunogenic. If, however, the small molecule is chemically linked to a large protein molecule, completely new epitopes will be formed on the surface of the larger molecule ([Figure 7-6](#)). If this molecular complex is injected into an animal, immune responses will be triggered against all its epitopes. Some of the antibodies made in response to the complex will be directed against new epitopes formed by the small molecule. Small molecules or chemical groups that can function as epitopes only when bound to other larger molecules are called haptens (in Greek, *haptein* means “to grasp or fasten”). The antigenic molecule to which the haptens are attached is called the carrier. Many drug allergies occur because the drug molecules, although small, can bind covalently to normal body proteins and so act as haptens.

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By using haptens of known chemical structure, it is possible to study the interaction between antibodies and epitopes in great detail. For example, antibodies raised against one hapten can be tested for their ability to bind to other, structurally related molecules. Simple tests have shown that any alteration in the shape, size, or charge of a hapten alters its ability to bind to antibodies. Even very minor modifications to the shape of a hapten, such as the difference between stereoisomers, may influence its ability to be bound by an antibody. Since an enormous variety of potential haptens exists, and since each hapten can provoke its own specific antibodies, it follows that animals must be able to generate an extremely large variety of specific antibody molecules. This enormous diversity enables animals to successfully fight the multitude of pathogenic microbes.

7.6.2

Some Examples of Haptens

Although the concept of haptens and carrier molecules provides the basis for much of our knowledge concerning the specificity of the antibody response, haptens may also be of clinical importance. For example, the antibiotic penicillin is a small nonimmunogenic molecule. Once degraded within the body, however, it forms a very reactive “penicilloyl” group, which can bind to serum proteins such as albumin to form penicilloyl-albumin complexes ([Figure 7-7](#)). The penicilloyl hapten can be recognized as a foreign epitope in some individuals and so provokes an immune response.

FIGURE 7-5 A molecular model of an antigen. This is an important influenza virus antigen called the hemagglutinin. It consists of two chains, one of which is lightly drawn so that the details of the other can be seen. The irregular surface forms characteristic shapes that can be recognized by the cells of the immune system. Influenza virus constantly changes the shape of this molecule, and this is denoted by the color coding. (Courtesy Dr. Fabian Glaser.)

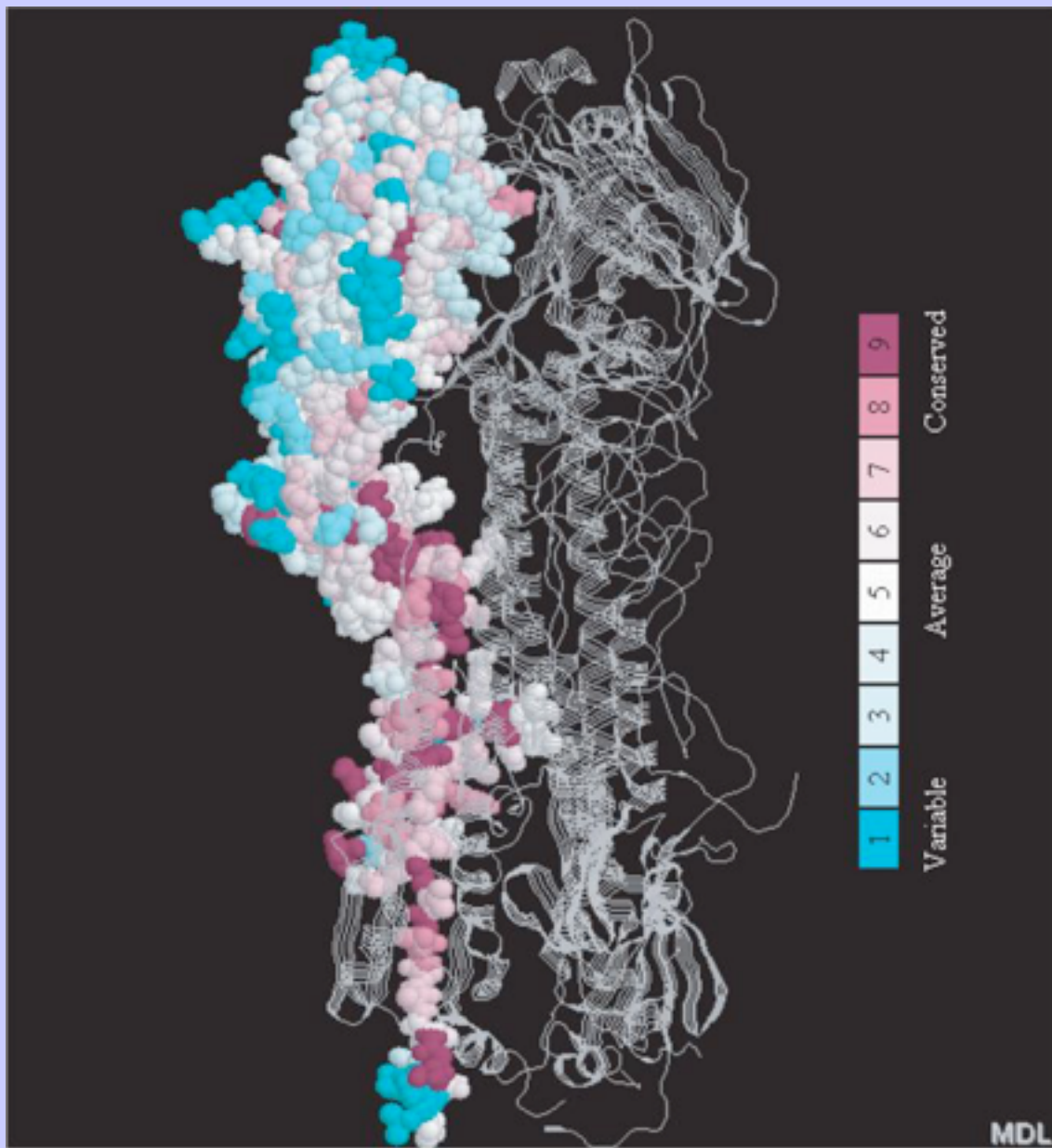
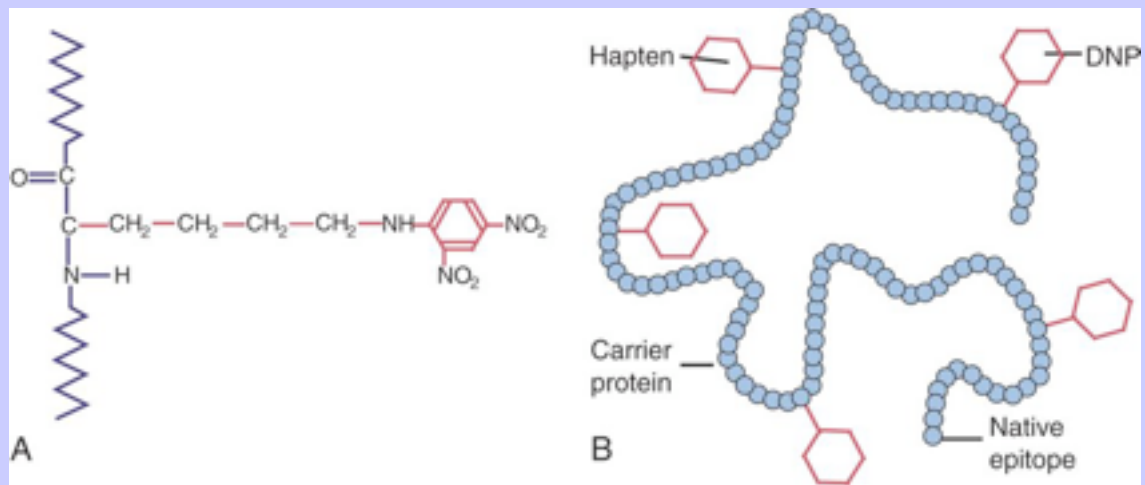


FIGURE 7-6 **A**, A typical hapten, in this case, dinitrophenol attached to a lysine side chain. **B**, When several haptens are attached to a peptide chain, they serve as new epitopes and will stimulate immune responses.



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FIGURE 7-7 Penicillin as a hapten. Penicillin can break down in vivo by several different pathways. The most important derivative is a penicillenic acid that combines with amino groups in a protein such as serum albumin to form a penicilloyl-protein complex. This complex may provoke an immune response and result in a penicillin allergy.

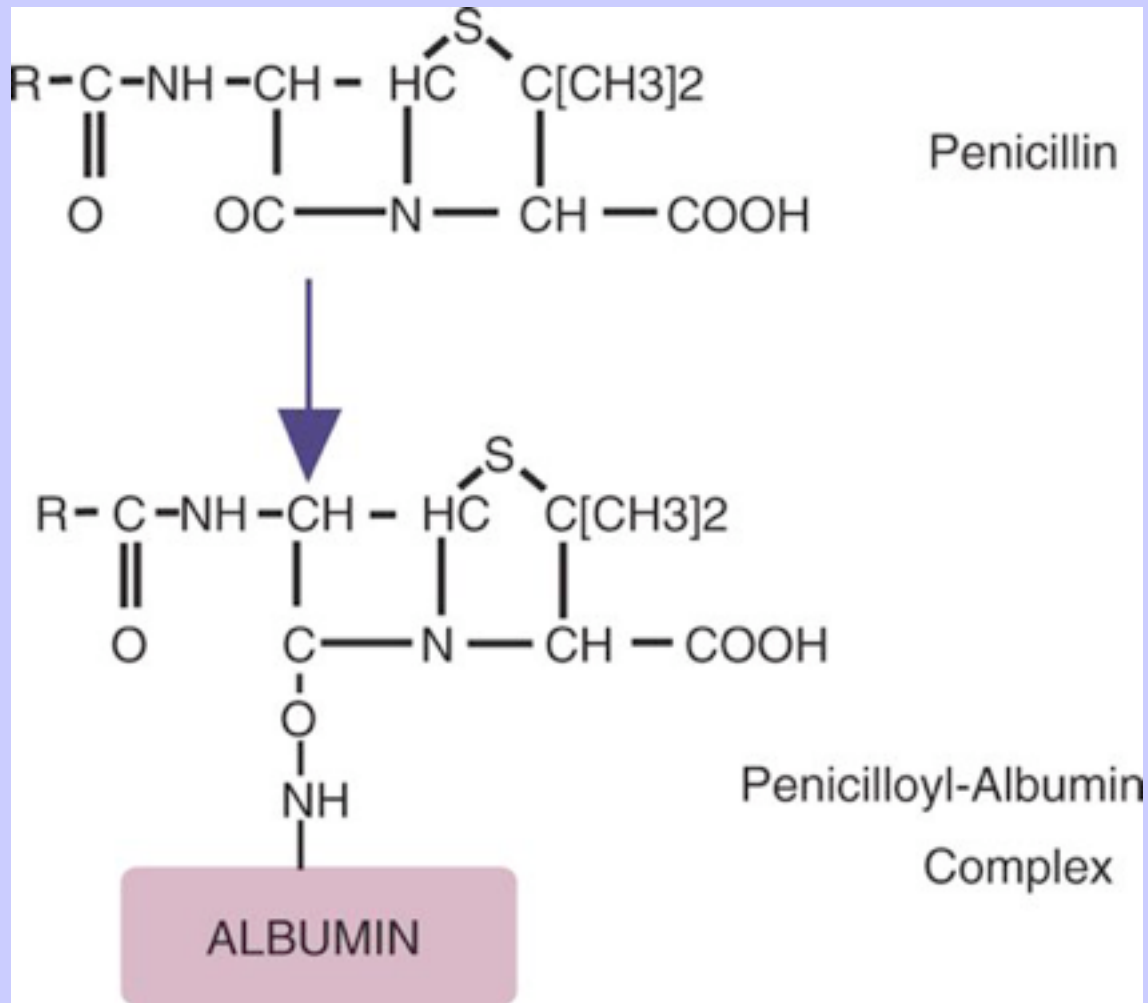
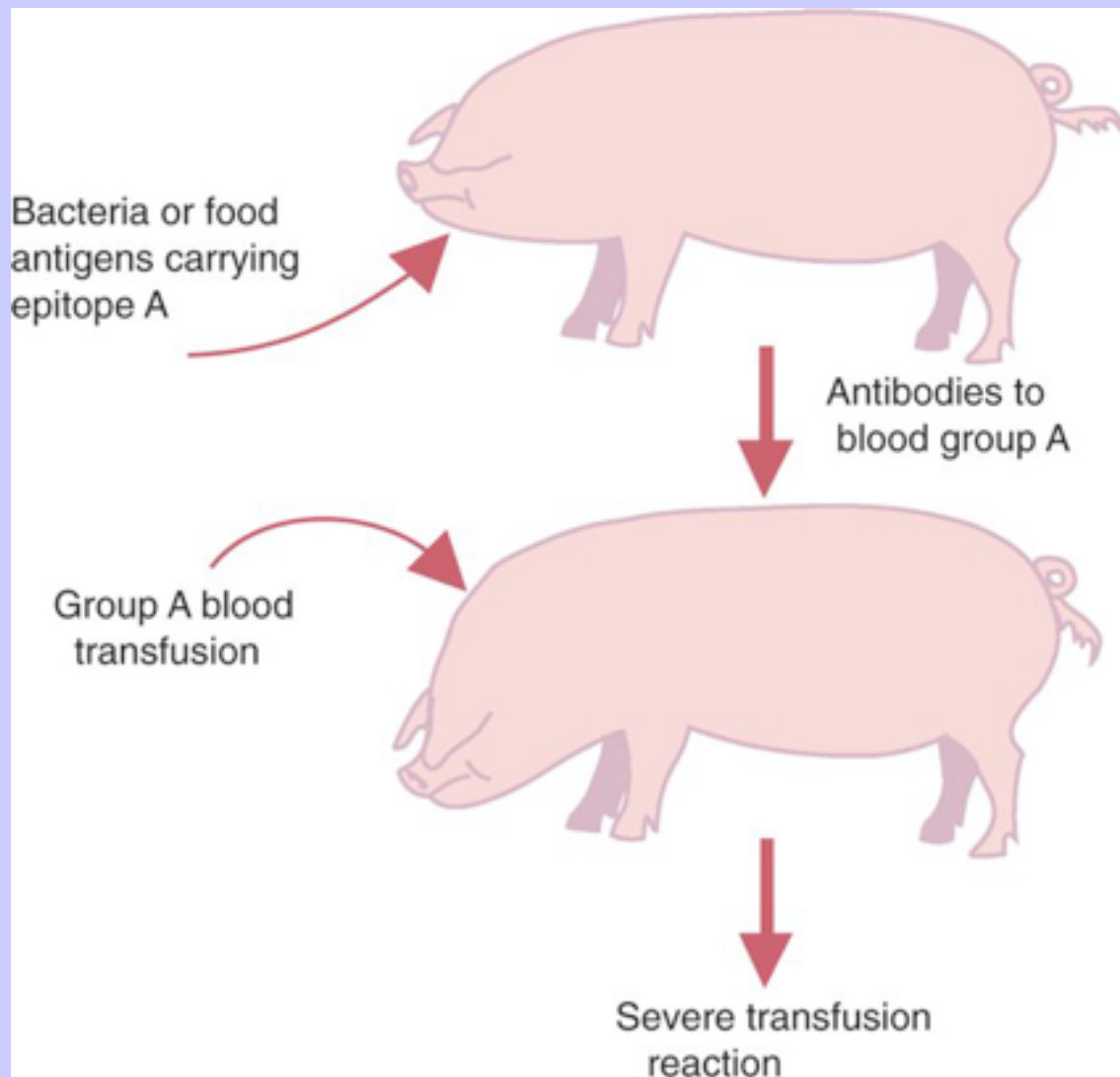


FIGURE 7-8 Food or bacterial antigens encountered in the diet carry epitopes that cross-react with blood group glycoprotein A. As a result, pigs of blood group O make antibodies to the A epitope despite never having received group A red cells. Should these animals be inadvertently transfused with A blood, they will suffer an immediate and severe transfusion reaction.



A second example of a naturally occurring reactive chemical that binds spontaneously to normal proteins and so acts as a hapten is the toxic component of the poison ivy plant (*Rhus radicans*). The resin of this plant, called urushiol, will bind to any protein with which it comes into contact—including the skin proteins of a person who rubs against the plant. The modified skin proteins are then regarded as foreign and attacked by lymphocytes in a

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manner similar to the rejection of a skin graft. The result is the uncomfortable skin rash called allergic contact dermatitis (see [Chapter 28](#)).

7.7 CROSS-REACTIONS

Identical or similar epitopes may sometimes be found on apparently unrelated molecules. As a result, antibodies directed against one antigen may react unexpectedly with an unrelated antigen. In another situation, the epitopes on a protein may differ in only minor respects from those on the same protein obtained from an animal of a related species. Consequently, antibodies directed against a protein in one species may also react in a detectable manner with the homologous or similar protein in another species. Both phenomena are called cross-reactions.

An example of a cross-reaction of the first type is seen when blood-typing. Many bacteria possess cell wall glycoproteins with carbohydrate side chains that are identical to those found on mammalian red blood cell glycoproteins. For example, some intestinal bacteria possess glycoproteins with A or B side chains on their cell walls (see [Chapter 26](#)). These glycoproteins are absorbed through the intestinal wall into the bloodstream and trigger an antibody response. For example, blood group glycoprotein side chain A is foreign to a pig of blood group O ([Figure 7-8](#)). Pigs of blood group O therefore develop antibodies that react with red cells from pigs of blood group A. These antibodies arise not as a response to previous immunization with group A red cells but following exposure to the glycoproteins that originate in the intestinal bacteria. Cross-reacting antibodies of this type are called heterophile antibodies.

Another example of cross-reactivity occurs between *Brucella abortus* and some strains of *Yersinia enterocolitica*. *Y. enterocolitica*, a relatively unimportant organism, may provoke cattle to make antibodies that cross-react with *B. abortus*. Since *Brucella*-infected animals are detected by testing for the presence of serum antibodies, a *Yersinia*-infected animal may be wrongly thought to carry *B. abortus* and so be killed. In another example, cross-reactivity occurs between the virus of feline infectious peritonitis (FIP) and the virus of pig-transmissible gastroenteritis (TGE). It is very difficult to grow the FIP virus in the laboratory. TGE virus, on the other hand, is readily propagated. By detecting antibodies to TGE in cats, it is possible to diagnose FIP without having to culture the FIP virus.

The second type of cross-reactivity, which occurs between related proteins, may be demonstrated in many different biological systems. One example is the method used to detect relationships between mammalian species. Thus antisera to bovine serum albumin cross-react well with sheep and goat serum albumin but react much more weakly with serum albumin from other mammals ([Table 7-1](#)). Presumably, this reflects the degree of structural similarity between the epitopes on serum proteins and is thus a useful tool in determining evolutionary relationships.

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Table 7-1 The Degree of Cross-Reaction between a Specific Antibody (Antibovine Light Chain Antibodies) and Related Proteins (Light Chains) from Other Mammals

Cow	<i>Bos taurus</i>	100
Bison	<i>Bos bison</i>	100
Sheep	<i>Ovis aires</i>	100
Yak	<i>Pocphagus grunniens</i>	68
Goat	<i>Capra hircus</i>	68
Elk	<i>Cervus canadensis</i>	64
Buffalo	<i>Bubalus bubalus</i>	54
Reindeer	<i>Rangifer tarandus</i>	37
Human	<i>Homo sapiens</i>	17
Horse	<i>Equus caballus</i>	10
Rat	<i>Rattus rattus</i>	10
Mouse	<i>Mus musculus</i>	10
Pig	<i>Sus scrofa</i>	8
Camel	<i>Camelus dromedarius</i>	7
Data from Henning D, Nielsen K: <i>Vet Immunol Immunopathol</i> 34:235-243, 1992.		

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8 CHAPTER 8 Dendritic Cells and Antigen Processing

8.1 KEY POINTS

- Certain cell types, namely dendritic cells (DCs), macrophages, and B cells, are able to capture and process antigen so that it can trigger acquired immune responses.
- The most effective of these antigen-presenting cells are DCs. Only DCs can effectively stimulate naïve T cells.
- Immature DCs are scattered throughout the body. They are especially equipped to capture foreign antigens.
- Once stimulated by foreign antigens, DCs mature and become highly effective in presenting these foreign antigens to T cells. In order to do this they express high levels of antigen receptors called major histocompatibility complex (MHC) class II molecules.
- DCs ingest foreign antigens, break them into small fragments, and then present them on their surface MHC molecules, where they can be recognized by T cells.
- Macrophages are also antigen-presenting cells, but since they also destroy ingested antigens, they are much less efficient than DCs.
- B cells are also antigen-presenting cells and are highly effective during a secondary immune response.
- There are two major subpopulations of DCs: myeloid and plasmacytoid. Plasmacytoid DCs are major producers of type I interferons.

The innate immune defenses are designed to destroy microbial invaders as soon as they enter the body. Most invaders, especially if they are of low virulence, are rapidly eliminated. However, in addition to being uncomfortable and damaging, inflammation is not a foolproof process. If the body is to be defended effectively, an animal must have defenses that automatically detect and eliminate all microbial invaders without the damage and discomfort associated with inflammation. This is the task of the acquired immune system.

In order to trigger acquired immunity, a sample of foreign material must be captured, processed, and presented in the correct fashion to cells that can recognize it. The first steps in this process are the responsibility of antigen-processing cells.

Antigen-processing cells are attracted by alarmins and activated by the same stimuli that trigger inflammation. Indeed, dendritic cells (DCs) and macrophages are both sentinel cells for innate immunity as well as effective antigen-processing cells. As a result, antigen processing can proceed at the same time as the invader is being eliminated by the innate defenses. Once an invader has been eliminated, the body can proceed to strengthen its defenses against a second attack by the same organism.

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Antigen processing involves breaking antigen molecules into small peptides. These peptides are then attached to specialized antigen-presenting receptors called major histocompatibility complex (MHC) molecules. There are two different classes of MHC molecules, MHC I and MHC II. Each binds a different type of antigen. Antigenic peptides bound to MHC are carried to the cell surface. Acquired immunity is triggered when these MHC-bound antigen peptides bind to specific receptors on lymphocytes. These lymphocytes (called T cells) respond only to antigen

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peptides that have been correctly processed. This helps ensure that acquired immune responses do not proceed indiscriminately.

The organisms that trigger acquired immunity are of two distinct types. One type includes the foreign microorganisms such as bacteria that invade the body from outside and grow in the tissues and extracellular fluid. Their antigens, called exogenous antigens, are processed by specialized antigen-processing cells. A second type of invading organism is made within the body. For example, when viruses invade a cell, they force the cell to make new viral proteins. These new proteins are called endogenous antigens and are processed by the cells in which they are generated.

Three cell types are employed to sample and process exogenous antigens: DCs, macrophages, and B cells. The most effective, by far, are DCs ([Figure 8-1](#)).

8.2 DENDRITIC CELLS

DCs perform three key functions. First, they serve as sentinel cells and so activate innate defenses. Second, they process exogenous antigen very efficiently to initiate acquired immune responses. Finally, they regulate both forms of immune response.

DCs are at least 100 times more potent as antigen-presenting cells than are macrophages or B cells. DCs can take up many different antigens—including dead microorganisms, soluble antigens in tissue fluids, and antigens released by dying cells—and present them to the cells of the immune system. DCs are the only antigen-processing cells that can activate T cells that have never previously encountered an antigen (naïve cells) and thus are essential for initiating primary immune responses.

8.2.1 Origin

DCs are produced by bone marrow stem cells. These immature DCs then migrate throughout the body and form lattice-like networks in virtually every tissue. DCs are found in all organs except the brain, parts of the eye, and the testes. They are especially prominent in lymph nodes, skin, and mucosal surfaces, where invading microbes are most likely to be encountered.

8.2.2 Structure

DCs have a variable morphology depending upon their state of activation. Typically, however, they have a small cell body with many long cytoplasmic processes known as dendrites ([Figure 8-2](#)). It is believed that the

FIGURE 8-1 The three major populations of antigen-presenting cells: B cells, dendritic cells (DCs), and macrophages. Of these, only DCs can activate naïve T cells and trigger a primary immune response.

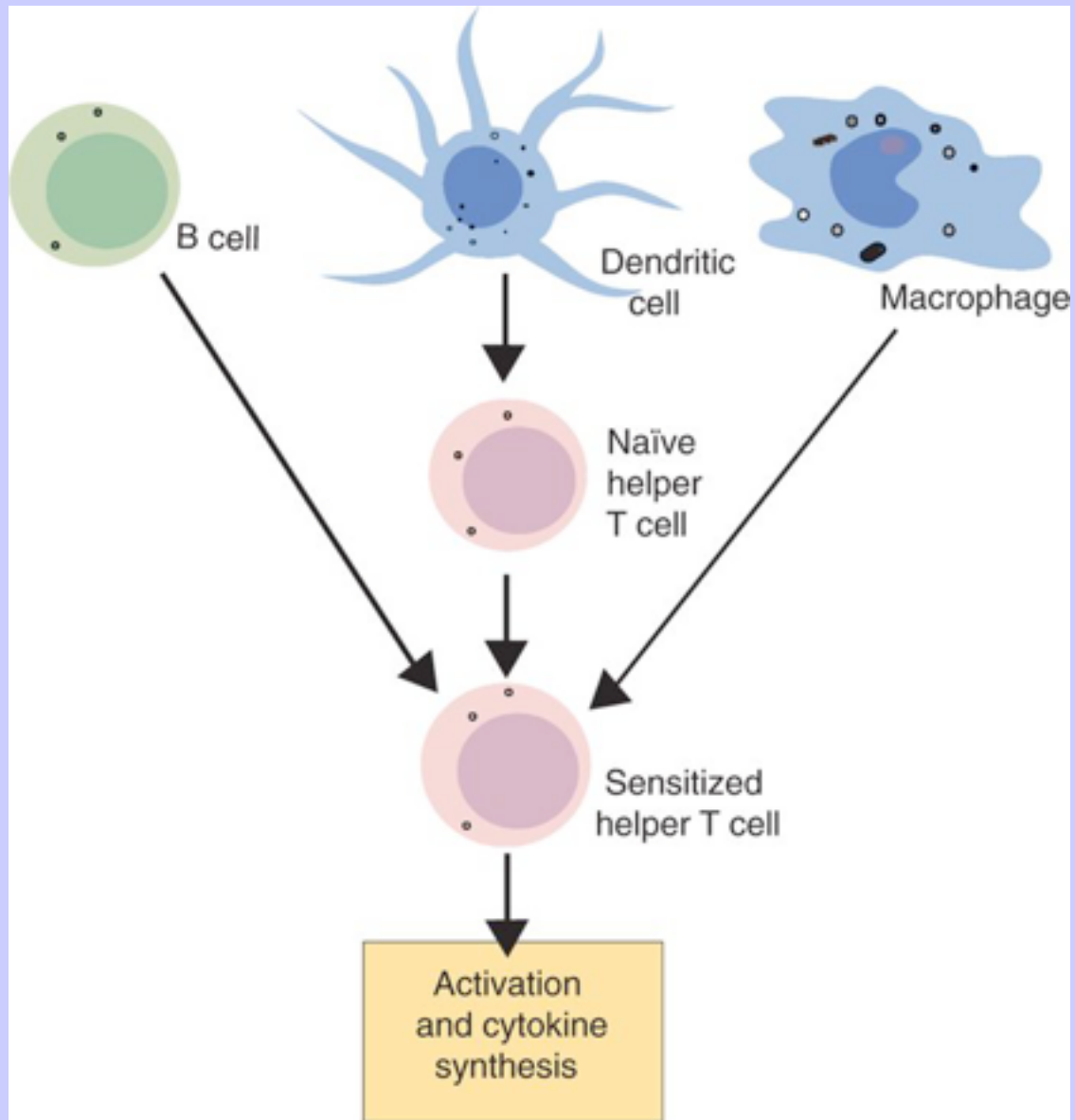
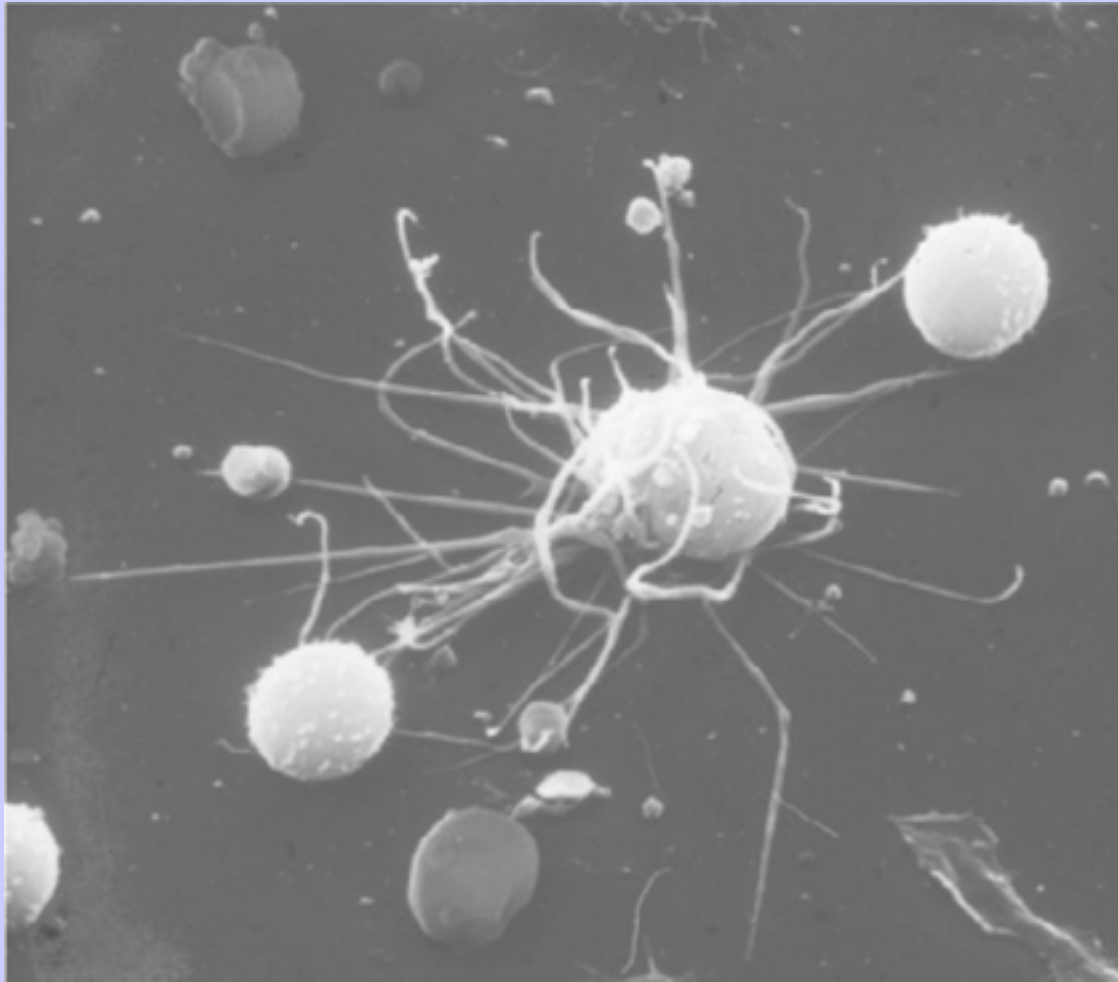


FIGURE 8-2 A scanning electron micrograph of a dendritic cell from a guinea pig lymph node. Note the relatively small cell body and the numerous long dendrites ($\times 4000$).



dendrites increase the efficiency of antigen trapping and maximize contact between DCs and other cells.

8.2.3

Subpopulations

DCs are a mixture of cell populations with variable functions. They are divided into two major populations called myeloid DCs (MDCs) and plasmacytoid DCs (PDCs) ([Figure 8-3](#)). These two populations differ in morphology, in surface antigens, and in function, although they share adhesion molecules, co-stimulatory molecules, and activation markers. MDCs are tissue DCs derived from blood monocytes. PDCs, in contrast, are found in blood and lymphoid organs and are derived from lymphoid precursors. On exposure to viruses, PDCs produce massive amounts of type I interferons (interferon- α [IFN- α] and IFN- β). Their numbers increase during infection. It is possible that the PDCs serve as an early warning system for viral infections since they are rapidly activated by microbial DNA.

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In mice two MDC populations are recognized. One expresses CD8a while the other does not. Mice also possess PDCs. These subpopulations consist of subsets of cells that differ in their surface markers, in their tissue location, in the cytokines they secrete, and in their functions.

8.2.3.1

Langerhans Cells

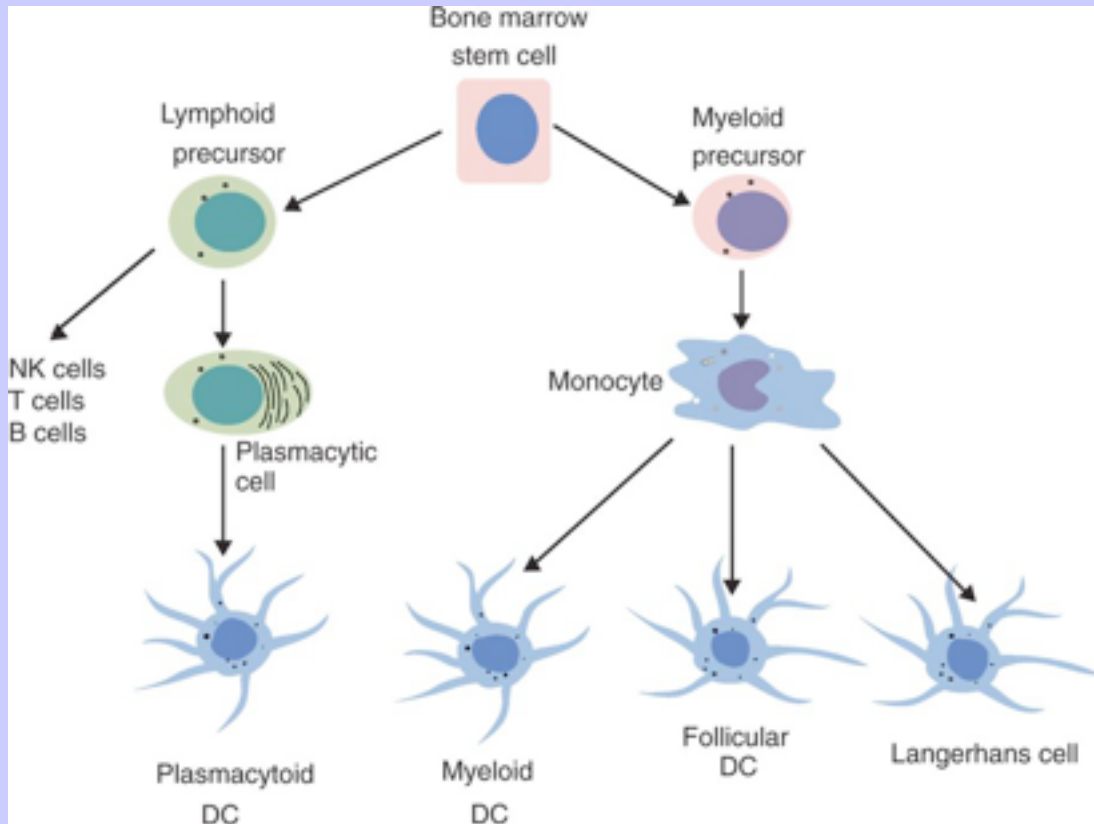
Langerhans cells are a specialized population of long-lived (18 month) MDCs found in the epidermis, where they trap and process antigens that penetrate the skin from outside ([Figure 8-4](#)). These include topically applied antigens, such as the resins of poison ivy, or intradermally injected antigens, such as mosquito saliva. Langerhans cells influence the development of skin immune responses, such as delayed hypersensitivity and allergic contact dermatitis (see [Chapter 28](#)). Langerhans cells contain characteristic cytoplasmic granules called Birbeck granules.

A specialized population of MDCs in the thymus may recognize self-reactive T cells and induce unresponsiveness in immature T cells (see [Chapter 17](#)). Some of these cells may express CD95L (CD178) and so stimulate T cell apoptosis. These DCs may develop from a lymphoid precursor since their production is driven by interleukin-3 (IL-3) and they lack myeloid cell markers.

Blood monocytes are the immediate precursors of both tissue macrophages and myeloid DCs, including Langerhans cells. Which specific cell type is produced depends on the cytokines and cells encountered by the monocyte as it matures. Each cell type can convert to the other until late in the differentiation process. In domestic animals myeloid DCs have been characterized in pigs, cattle, horses, chickens, and dogs while Langerhans cells have been described in pigs, cattle, horses, dogs, and cats. Plasmacytoid DCs have been identified in pigs.

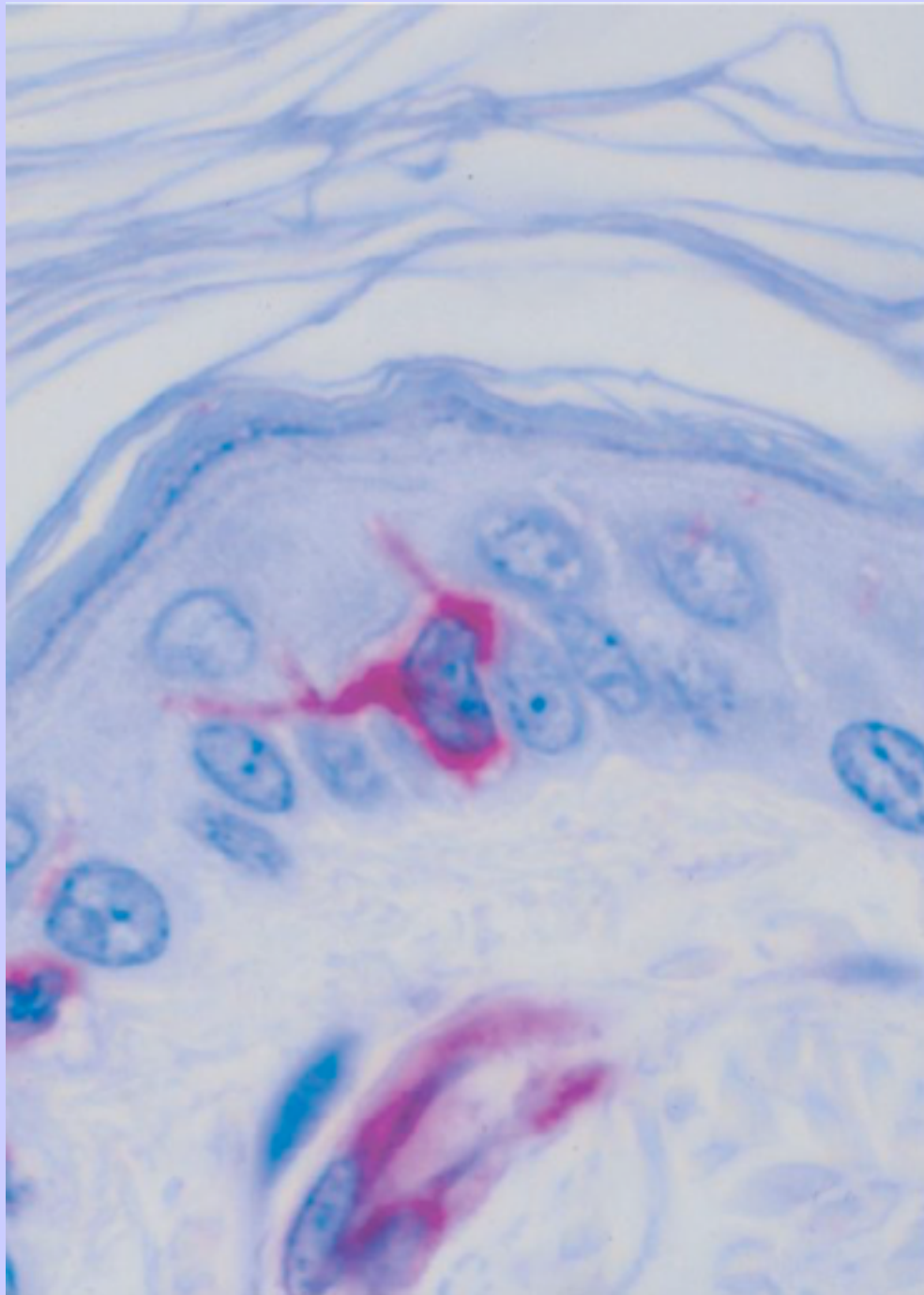
Although different populations of DCs have been characterized, the most important division is based on their state of maturity ([Figure 8-5](#)). Thus immature DCs are highly specialized and efficient antigen-trapping

FIGURE 8-3 The origins of dendritic cells (DC). One population, the plasmacytoid DCs, originates from lymphoid precursors. These give rise to DC2 type cells. The second major population arises from myeloid precursors and is closely related to monocytes. These give rise to DC1 cells.



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FIGURE 8-4 This dark red cell in the epidermis of a dog is a Langerhans cell stained for the protein vimentin. Note that its dendrites extend between the epidermal cells so that it can effectively trap antigens. (Courtesy Dr. K.M. Credille.)



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cells. As they mature, DCs undergo cellular reorganization and become specialized and efficient antigen-processing cells.

8.2.3.2

Immature Dendritic Cells

Newly generated MDCs migrate from the bone marrow through the blood to the lymph and eventually to lymph nodes or tissues. Here they serve as “sentinels” whose role is to capture invading microbes. With their short life span, they can be regarded as disposable antigen-trapping cells. If they do not encounter antigens, they die in a few days. If they encounter antigens together with tissue damage or inflammation, they become activated and mature rapidly. DCs have many different surface receptors that help them carry out their functions. These include cytokine receptors such as IL-1R and tumor necrosis factor receptor (TNFR), chemokine receptors, C-type lectins, Fc receptors (FcγR and FcεR), mannose receptors (CD206), heat shock protein receptors, and toll-like receptors (TLRs).

DC maturation occurs in response to alarmins such as IL-1 and TNF-α and pathogen-associated molecular patterns (PAMPs) such as lipopolysaccharides or microbial nucleic acids. Thus injured and inflamed tissues release large amounts of soluble heparan sulfate that binds to TLR4 and activates DCs. Breakdown of nucleic acids generates uric acid, another potent DC activator. One of the most important activators of immature DCs is high mobility group box protein-1 (HMGB1). HMGB1 also induces the migration of these cells to sites of inflammation and microbial invasion. Immature DCs are attracted to areas of inflammation by chemokines and defensins.

Immature DCs specialize in capturing antigens and cell fragments by phagocytosis, by pinocytosis (the uptake of fluid droplets, or “cell drinking”), and by interaction with various cell surface receptors. They also capture apoptotic cell bodies. If they ingest bacteria, they can usually kill them. They can distinguish between normal tissue debris and foreign organisms by selectively sampling their environment. This differentiation depends on the ability of the foreign material to bind to TLRs. Activation of TLRs by microbial components ensures that ingested material is processed in such a way that it triggers acquired immunity. Material that does not activate TLRs is not processed and so will not trigger an immune response.

While the phagosomes of conventional phagocytic cells such as neutrophils and macrophages are very acidic and hence optimal for proteolytic destruction of foreign material, the pH of DC and B cell phagosomes is relatively alkaline since the phagosomes of DCs fail to fuse with acidic granules. Cysteine and aspartyl proteases are inhibited at these high pH levels and antigen is preserved for cross-presentation on MHC class I molecules. Antigen therefore persists within these cells for long periods.

8.2.3.3

Mature Dendritic Cells

Upon encountering an antigen, DCs mature rapidly and enhance their antigen-presenting abilities. After they have captured and processed antigens, immature DCs carry the antigen to sites where it can be recognized by T cells. These activated DCs are attracted to lymphoid organs by the chemokine CCL20. Infection or tissue damage also promotes the migration of antigen-bearing DCs to lymph nodes or the spleen. Once they enter a lymphoid organ, they mature ([Figure 8-6](#)).

Mature DCs secrete the chemokine CCL22. This attracts T cells, which consequently accumulate in clusters around the DC. The DCs wrap the T cells in a net of dendrites as they interact. The T cells examine the mature DCs for the presence of antigen fragments. T cells will bind these fragments only if their antigen receptors match.

As DCs mature, their MHC molecules move from intracellular endosomes and lysosomes to the cell surface.
Cell surface expression of co-stimulatory

92

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FIGURE 8-5 As dendritic cells (DCs) mature, they change their function. Immature DCs are specialized antigen-trapping cells. Mature DCs, on the other hand, are specialized antigen-processing cells.

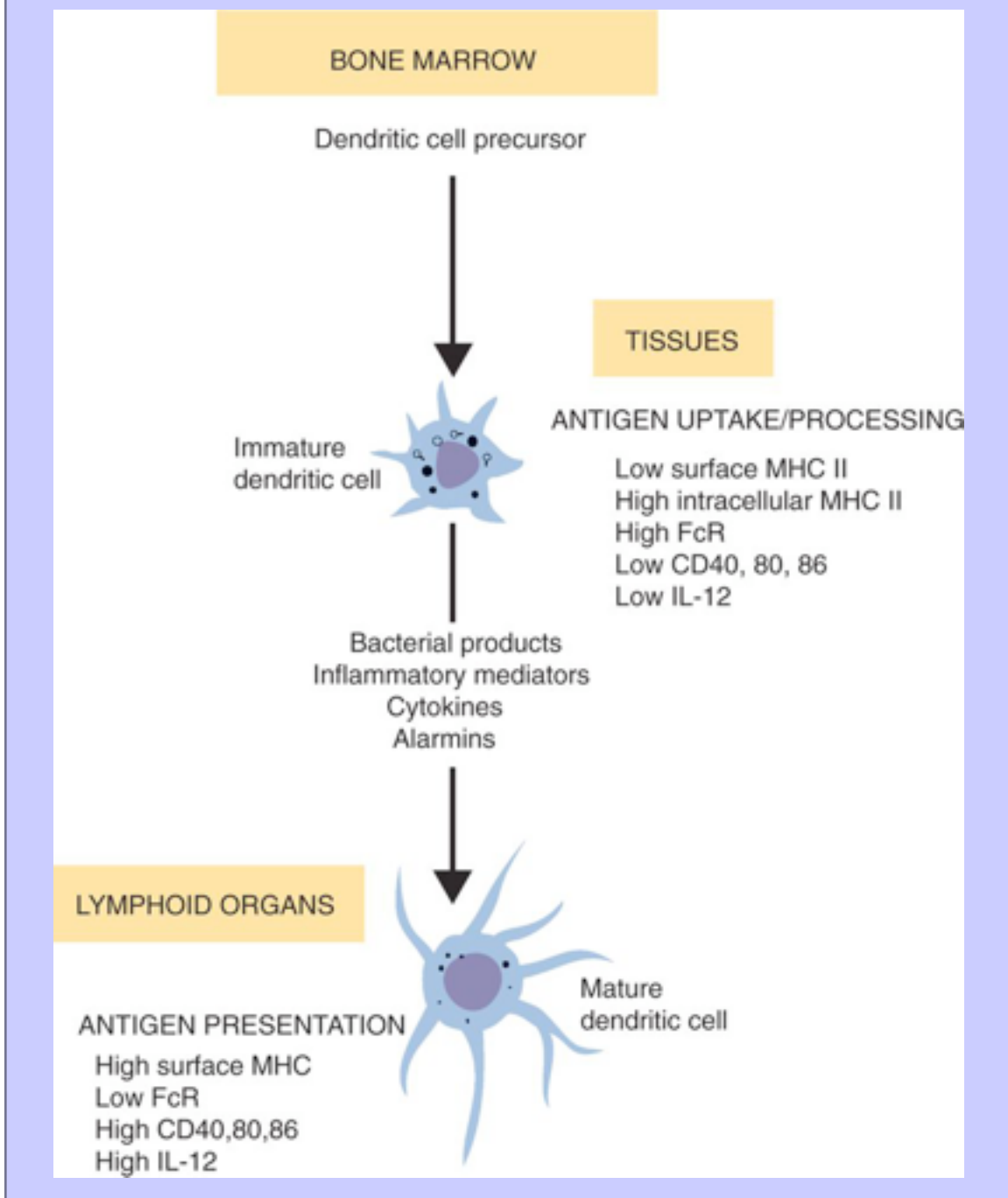
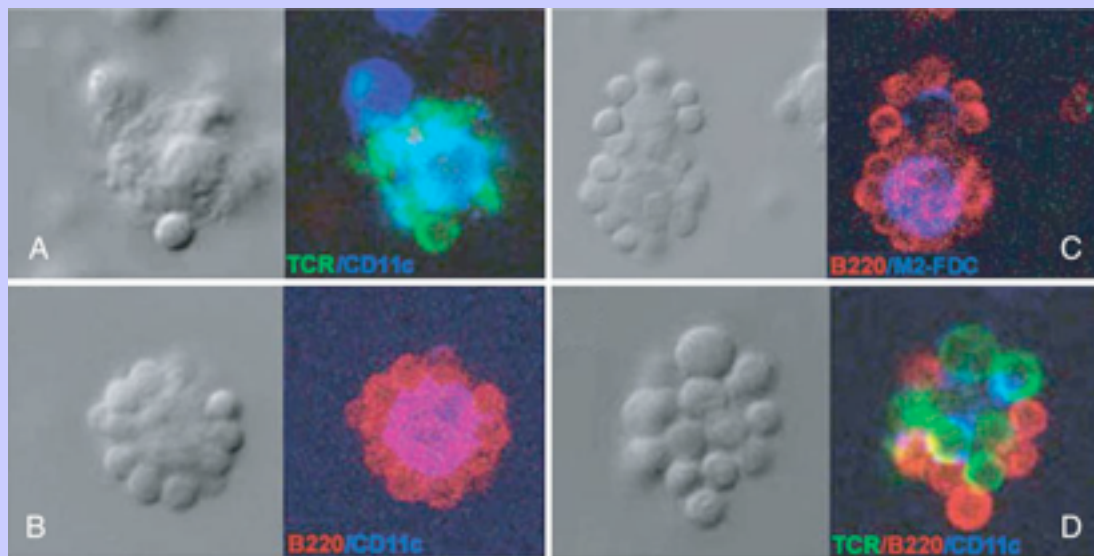


FIGURE 8-6 When dendritic cells (DCs) and T cells interact, they form visible clusters as the cells converse among themselves. Thus in these figures, DCs are stained with a blue fluorescent dye (anti-CD11c), T cells are stained with a green dye (anti-CD3), and B cells are stained with a red dye (anti-B220). **A**, T cells are interacting with a dendritic cell (DC). **B**, B cells are binding to a DC. **C**, B cells are binding to a follicular DC. **D**, There is a mixed B and T cell cluster. Note that some B cells appear to be attached to T cells. (From Hommel M, Kyewski B: *J Exp Med* 197:269-280, 2003.)



molecules also increases. As a result, MHC molecules and MHC-peptide complexes are found at levels 100 times higher on DCs than on other antigen-processing cells such as B cells or macrophages, and their expression of co-stimulatory molecules such as CD86 (see [Chapter 12](#)) may also rise 100-fold. Fc and mannose receptors, in contrast, are downregulated, and endocytosis is reduced.

Mature DCs are the only cells that can trigger a primary T cell response. One reason for this efficiency is that these cells may preassemble a complete T cell activation complex (antigen-loaded MHC plus co-stimulatory molecules) within the cell before it arrives at the cell surface. Mature DCs express DC-SIGN, a C-type lectin, which binds its ligand intercellular adhesion molecule-3 on naïve T cells. DC-SIGN thus mediates transient binding between DCs and T cells. It permits a single DC to screen thousands of T cells in order to find the few that are expressing a compatible antigen receptor. Because of their potency, only a few DCs are needed to trigger a strong T cell response. Indeed, one DC may activate as many as 3000 T cells.

While the most important function of DCs is to trap, process, and present antigen to the cells of the immune system, they must also be able to kill any pathogens they encounter. Thus DCs express nicotinamide

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adenosine dinucleotide phosphate oxidase and can kill invading organisms by mounting a respiratory burst. Exposure to PAMPs that activate TLRs enhances the production of superoxide by DCs.

8.2.3.4

Plasmacytoid Dendritic Cells

Plasmacytoid DCs are the major producers of type I interferons and so have a unique ability to link innate and acquired immunity. After producing large amounts of type I interferon, they are still able to differentiate into mature DCs that can stimulate naïve T cells. Because PDCs secrete large amounts of IFN- α , they activate NK cells. IFN- α secreted by PDCs can also initiate activation, enhance long-term survival, and promote the differentiation of T cells. They can also activate some B cells. PDCs secrete and are activated by HMGB1.

8.2.4

Tolerance Induction

Under steady state conditions—in the absence of inflammation, alarmins, or PAMPs—some immature DCs spontaneously mature and migrate to lymphoid tissues carrying tissue antigens with them. If a T cell recognizes these “normal” antigens, it may undergo apoptosis and die. Alternatively these DCs may trigger the production of IL-10–producing regulatory T cells. Thus the processing of normal body components by DCs leads to T cell deletion and immunological tolerance. The spontaneously mature DCs may present T cells with a unique signal, or perhaps they will deliver a signal that is insufficient to trigger a full T cell response

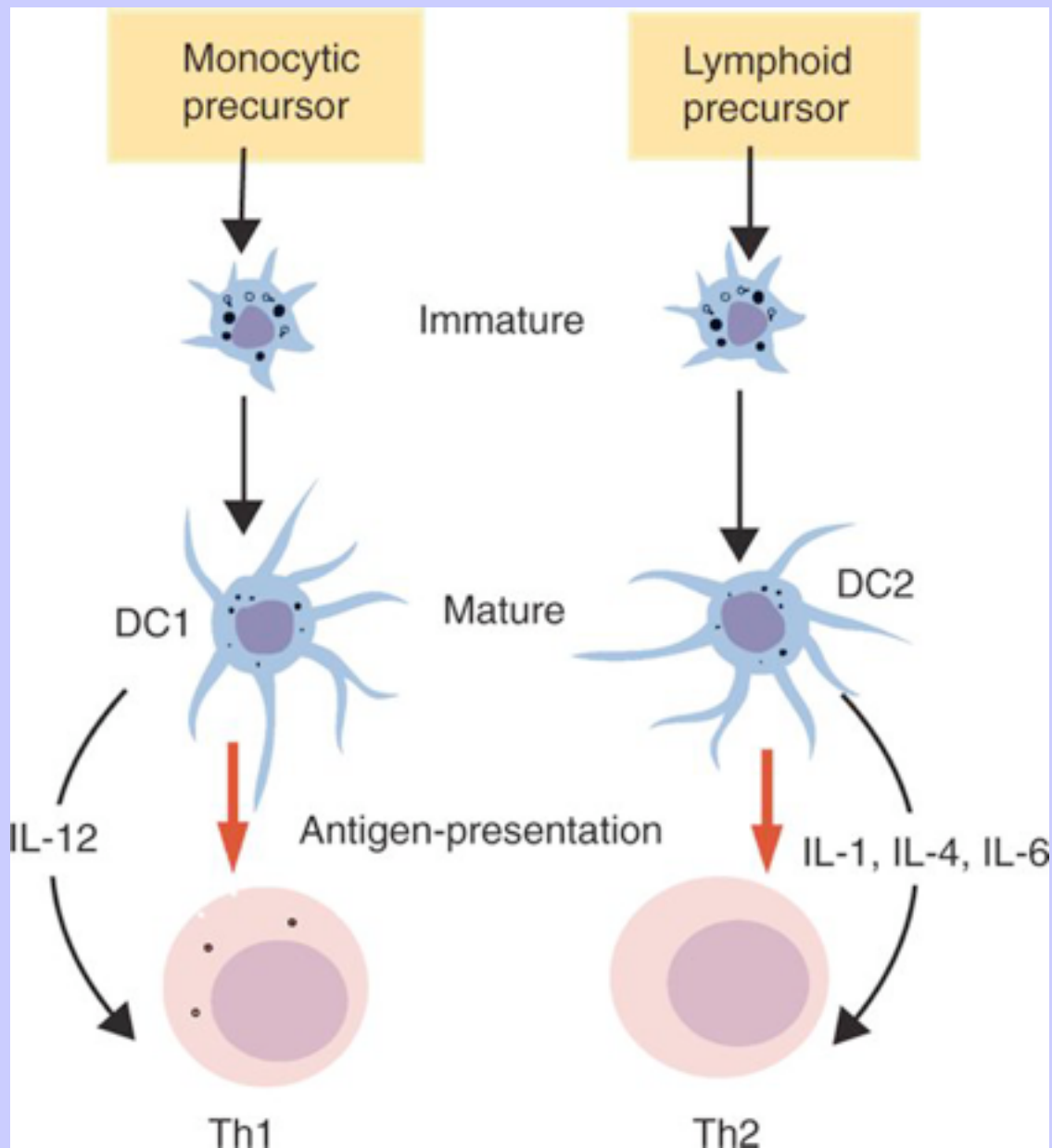
8.2.5

DC1 and DC2 Cells

As pointed out earlier, the acquired immune system has two major branches, the antibody-mediated and cell-mediated immune responses. The type of immune response mounted by an animal is determined by the type of helper T (Th) cell triggered to respond to an antigen. Thus there are two subpopulations of Th cells ([Figure 8-7](#)). One subpopulation, called Th1 cells, stimulates cell-mediated immune responses designed to protect animals against intracellular organisms. The other subpopulation, Th2 cells, stimulates antibody-mediated immune responses designed to protect animals against extracellular invaders. Which T cell population is activated depends on the use of distinct DC subpopulations.

When DCs stimulate T cells, they provide three signals. The first signal is delivered by contact with an antigen fragment associated with an MHC molecule. The second signal provides co-stimulation through molecules such as CD40 and CD80/86. The third signal determines the way in which naïve Th cells will develop. The nature of this third signal is determined

FIGURE 8-7 Two subpopulations of dendritic cells (DC) appear to favor different T cell subpopulations. Th1 cells promote cell-mediated immunity, whereas Th2 cells promote antibody formation. The helper cell population employed depends on the use of DC subpopulations. These cells have different origins and secrete different co-stimulatory cytokines.



by the conditions under which the DCs are activated. Some microbial molecules promote DC secretion of IL-12. These are called DC1 cells since their IL-12 activates Th1 cells. In contrast, other microbial molecules induce DCs to secrete IL-1, IL-6, and IL-4. Because these cytokines stimulate Th2 cells, IL-4-producing DCs are called DC2 cells. Some microbial molecules and alarmins induce DCs to secrete IL-23 and so stimulate Th17 cells.

Different PAMPs and alarmins acting through different TLRs determine the development of specific DC subpopulations. The stimuli that promote a DC1 response include dsRNA acting through TLR3, lipopolysaccharide acting through TLR4, flagellin acting through TLR5, and nucleic acids acting through TLR7 and TLR9. On the other hand, inflammatory mediators (e.g., IL-10, TGF- α , prostaglandin E₂, histamine, extracts of the parasitic worm *Schistosoma mansoni*, or the toxin of *Vibrio cholerae*) promote DC2 responses. DC2 responses are also triggered by lipopolysaccharides, proteoglycans, and zymozan (yeast) acting through TLR2, TLR6, and TLR1. As pointed out previously, a similar functional division occurs in macrophages. Thus M1 cells have a distinctly different metabolic program than M2 cells. These two populations, when acting as antigen-presenting cells, have differential effects on Th cells. Ligands of TLR2 promote the production of IL-23 but not of IL-12.

It may be that the same DC can promote a Th1, Th2, or Th17 response, depending on the dose and type of antigen it is exposed to. The response may also depend on the location of the DCs when they encounter the antigen. For example, DCs from the intestine or airways seem to preferentially secrete IL-10 and IL-4 and thus promote Th2 responses. In those cases, the intestinal microenvironment provides the DC-polarizing signals.

8.2.6

Follicular Dendritic Cells

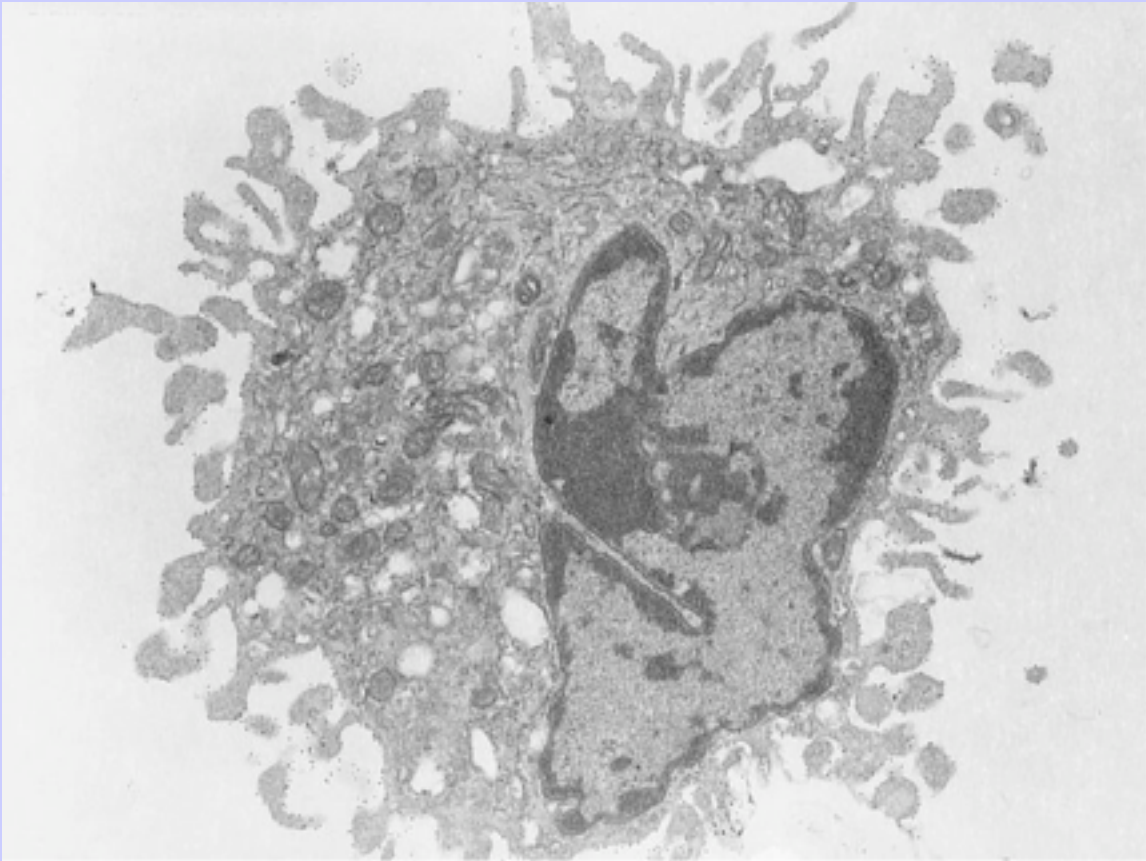
Specialized DCs found in lymphoid follicles trigger B cell activation. They are a form of MDC derived from monocytes. These follicular DCs present antigens to B cells in two different ways. In an unprimed animal (i.e., an animal that has not previously been exposed to the antigen), antigen presentation is a passive process. The DCs simply provide a surface on which antigen can be presented. In animals that have previously been exposed to an antigen and so possess antibodies, the antigen and antibody combine to form antibody-antigen complexes (also called immune complexes). Follicular DCs take up these immune complexes on their surface and then shed them in round-beaded structures called exosomes. B cells take up these exosomes and, after processing the antigen, present it to antigen-sensitive T cells. Follicular DCs can retain antigens on their surface for more than 3 months.

8.3

DENDRITIC CELLS IN DOMESTIC ANIMALS

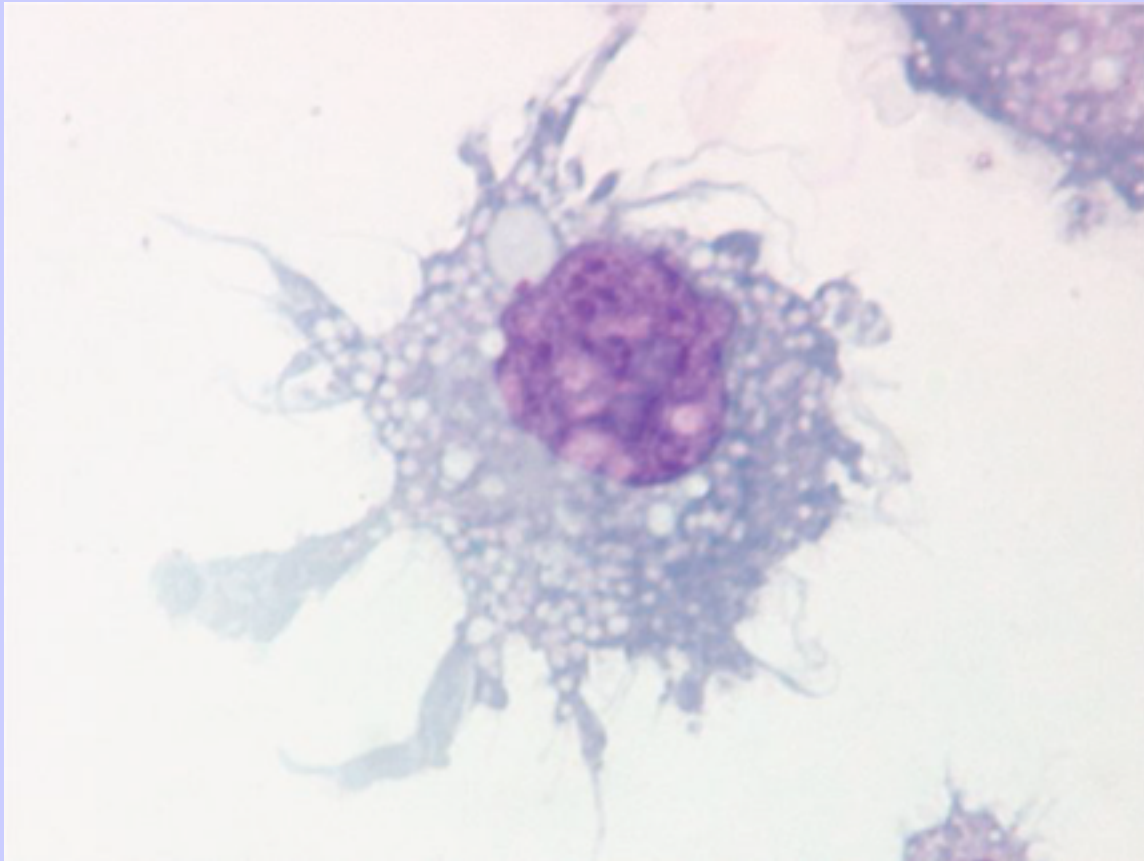
DCs have been identified in all the major domestic animal species. They do not appear to differ in any significant respect from DCs in humans and mice. For example, equine DCs are MHC class II⁺, CD11⁺, EqWC1⁺, and EqWC2⁺. Bovine DCs express not only CD80, CD86, and CD40 ([Figure 8-8](#)) but also other molecules restricted to the bovine. Cattle possess two major DC subpopulations that differ in their ability to stimulate CD4 and CD8 T cells. One population synthesizes more IL-12, whereas the other population produces more IL-1 and IL-10; these may well represent DC1 and DC2 subpopulations. DCs derived from sheep peripheral blood monocytes are MHC class II⁺, CD11c⁺, and CD14⁻. Pigs have both myeloid and plasmacytoid DCs. Pig MDCs are CD172a⁺, CD11R1⁺, CD1^{+/-}, and CD80/86^{+/-}, while their PDCs are CD172a⁺, CD4⁺, CD1^{+/-}, and CD80/86^{+/-}. Both types secrete IL-10 and IL-12. There are two main populations of canine DCs. One is MHC class II⁺, CD34⁺, and CD14⁻; the other is MHC class II⁺, CD34⁺, and CD14⁺. Feline Langerhans cells are CD18⁺, MHC class II⁺, CD1a⁺, and CD4⁺. DCs obtained from feline blood mononuclear cells are CD1⁺, CD14⁺, and MHC classes I and II⁺ ([Figure 8-9](#)).

FIGURE 8-8 A transmission electron micrograph of a dendritic cell from bovine afferent lymph. It has been stained with a monoclonal antibody specific for bovine CD1b. (The antibody is linked to colloidal gold particles, which are visible as small, electron dense dots around the outside of the cell.) (Courtesy Dr. C.J. Howard and Dr. P. Bland, Institute for Animal Health, Compton, United Kingdom.)



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FIGURE 8-9 A feline dendritic cell cultured in the presence of recombinant human interleukin-4 and granulocyte-macrophage colony-stimulating factor. Note the extensive dendrites so characteristic of these cells. Bright field illumination $\times 100$. (From Sprague WS, Pope M, Hoover EA: *J Comp Path* 15:139, 2005.)



8.4 OTHER ANTIGEN-PROCESSING CELLS

Naïve T cells require prolonged interaction with DCs if they are going to respond to antigens. Once primed, these T cells can be further activated by relatively transient interactions with antigen-presenting macrophages or B cells.

8.4.1 Macrophages

Macrophages are the most accessible and best understood of the antigen-processing cells. Their properties have been described in [Chapter 4](#). Once antigens are taken up by macrophages, a portion is processed and presented to sensitized T cells. Macrophages are, however, unable to engage in prolonged interactions with T cells. As a result, they cannot activate naïve T cells. In addition, antigen processing by macrophages is inefficient, since

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much of the ingested antigen is destroyed by lysosomal proteases and oxidants. Indeed, macrophages and B cells can be considered to be cells with other priorities (some call them “semiprofessional” antigen-processing cells).

8.4.2

B Cells

B cells, like macrophages, cannot undertake prolonged interactions with T cells. They do, however, have antigen receptors that enable them to bind and process large amounts of specific antigen. In association with MHC class II molecules, they ingest and process antigens before presenting them to sensitized T cells. B cells probably play a very minor role in antigen processing in a primary immune response but a much more significant one in a secondary response, when their numbers have greatly increased and T cells are easier to stimulate.

8.4.3

Other Cells

It has been generally believed that only the “professional” antigen-processing cells can stimulate T cell responses, since only these professional cells can load antigen fragments on their surfaces and provide the correct co-stimulatory signals. However, T cells may be activated by many different “nonprofessional” cell types. These include neutrophils, eosinophils, T cells, endothelial cells, fibroblasts, natural killer cells, smooth muscle cells, astrocytes, microglial cells, and some epithelial cells such as thymic epithelial cells and corneal cells. Their effectiveness may depend on the local environment. Thus fibroblasts can be very effective antigen-processing cells when located within lymphoid organs. Presumably co-stimulation can come from other nearby cells in this cytokine-rich environment. Vascular endothelial cells can also take up antigens, synthesize IL-1, and (under the influence of IFN- γ) express MHC class II molecules. Even skin keratinocytes can secrete cytokines similar to IL-1, express MHC class II molecules, and present antigen to T cells. In pigs, a subpopulation of circulating γ/δ T cells can act as professional antigen-processing cells.

8.5

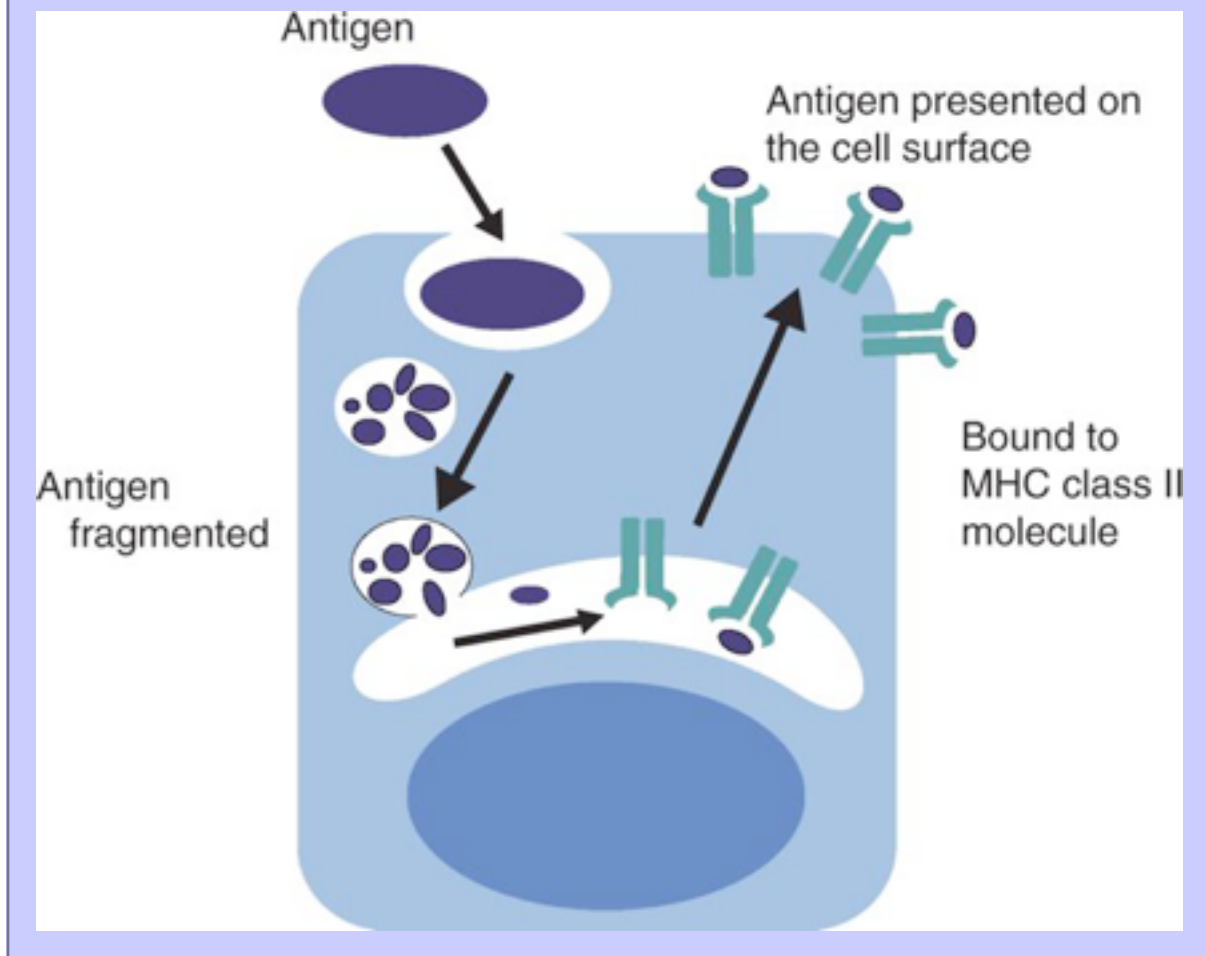
PROCESSING OF EXOGENOUS ANTIGEN

The presentation of exogenous antigen is controlled by MHC class II molecules. Although many cells can phagocytose foreign particles, only those that express antigen linked to MHC class II molecules can trigger an immune response. The most efficient antigen-processing cells are thus mature MHC II⁺ DCs. Unlike macrophages, DC lysosomes have limited proteolytic activity and degrade internalized antigens slowly. As a result, these antigens may persist for a long time inside DCs. MHC class II molecules can bind fragments of these ingested molecules and present them to Th cells ([Figure 8-10](#)). Th cells recognize and respond to fragments of foreign antigen only if they are bound to MHC class II molecules. If an antigen is presented to T cells without being linked to an MHC class II molecule, the T cells will be turned off or die, and tolerance may result (see [Chapter 17](#)).

Multiple steps occur in exogenous antigen processing. First, the antigen must be phagocytosed and taken into the phagosome. The phagosome then fuses with lysosomes containing proteases. Ingested peptides are broken down by these proteases into fragments of varying length. The endosomes containing these

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FIGURE 8-10 The processing of exogenous antigen by an antigen-presenting cell. Ingested antigens are taken into phagosomes, where they are fragmented by proteases. Peptides are then carried to the endosomal compartments, where the antigenic peptides are placed in the binding grooves of major histocompatibility complex (MHC) class II molecules. The complexes are then carried to the cell surface, where they are presented to helper T cells.



peptide fragments then fuse with other endosomes carrying newly synthesized MHC class II molecules.

Newly synthesized MHC class II chains are translocated to the endoplasmic reticulum, where they are assembled into a large complex together with a peptide called the invariant chain (Ii). This complex travels to an endosome, where the invariant chain molecules are digested, leaving a small peptide called class II-associated Ii peptide (CLIP), which remains associated with the MHC molecule. The CLIP chain occupies the antigen-binding site of the MHC molecule. When antigen-containing lysosomes fuse with the MHC-containing endosomes, the antigen peptides are exchanged for the CLIP chain. An MHC antigen-binding site can hold a peptide of 12 to 24 amino acids as a straight, extended chain that projects out of both ends of the binding site. Side chains from the peptide

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bind in pockets on the walls of the binding site. The presence of the CLIP chains prevents the endosomes containing the MHC class II molecules from being prematurely transported to the cell surface. Thus, unlike most transmembrane proteins that are expressed minutes after assembly, MHC class II molecules are retained inside the cell for several hours until they are needed.

Once the antigen fragment and MHC molecule have combined, the fused vesicles move toward the cell surface. Once they reach the cell surface, the vesicle fuses with the cell membrane and the MHC-peptide complex is presented on the cell surface.

It has been calculated that an antigen-processing cell carries about 2×10^5 MHC class II molecules that can present antigen fragments to T cells. If co-stimulation is provided, a T cell can be activated by exposure to 200 to 300 peptide-MHC complexes; it is therefore possible for one antigen-processing cell to present many different antigens simultaneously.

Since Th cells must be stimulated by MHC-antigen complexes for most immune responses to occur, MHC class II molecules effectively determine whether an animal can respond to any antigen. Class II molecules can bind some, but not all, peptides created during antigen processing, and in effect, select those antigens that are to be presented to T cells. (Further coverage of MHC molecules is provided in [Chapter 9](#).)

8.6

PROCESSING OF ENDOGENOUS ANTIGEN

One function of T cell-mediated immune responses is the identification and destruction of cells producing abnormal or foreign proteins. The best examples of such cells are those infected by viruses. Viruses take over the protein-synthesizing machinery of the cell and use it to make new viral proteins ([Figure 8-11](#)). To control infection, cytotoxic T cells must recognize the viral proteins expressed on the surface of infected cells. They will respond to these endogenous antigens only if they are processed and their fragments bound to an MHC class Ia molecule. Cytotoxic T cells recognize protein-MHC class Ia complexes. This can be demonstrated experimentally by showing that cytotoxic T cells can destroy virus-infected target cells only if the T cells recognize the MHC class Ia molecules expressed on the target cell (see [Chapter 16](#)). These cells are thus said to be MHC-restricted. The MHC class Ia chain is folded in such a way that a large antigen-binding site is formed on its outermost surface (see [Chapter 9](#), [Figures 9-5](#) and [9-6](#)). This binding site, however, differs from that on MHC class II molecules in that it is closed at each end with deep pockets. As a result, long peptides cannot project out of the ends of the site. Because the binding site is closed at each end, MHC class Ia molecules can only bind peptides containing nine amino acids. Indeed, in order to do this, these peptides must bulge out in the middle. Overall, however, the antigen-binding sites on class II and class Ia molecules function in a very similar manner.

The processing of endogenous peptides is very different from the processing of exogenous peptides. Living cells continually break down and recycle proteins. As a result, abnormal proteins are removed, regulatory peptides do not accumulate, and amino acids are made available for other purposes. As a first step, the lysines in protein molecules to be recycled are attached to ubiquitin, a 76-amino acid polypeptide

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FIGURE 8-11 The processing of endogenous antigen. Samples of newly synthesized proteins are ubiquitinated prior to being chopped into peptides by a proteasome. The peptides attach to a transporter protein located in the membrane of the endoplasmic reticulum. They are then carried into the lumen of the endoplasmic reticulum where they are placed in the antigen-binding groove of major histocompatibility complex (MHC) class I molecules. The MHC class I/peptide complexes are carried to the cell surface where they encounter cytotoxic T cells.

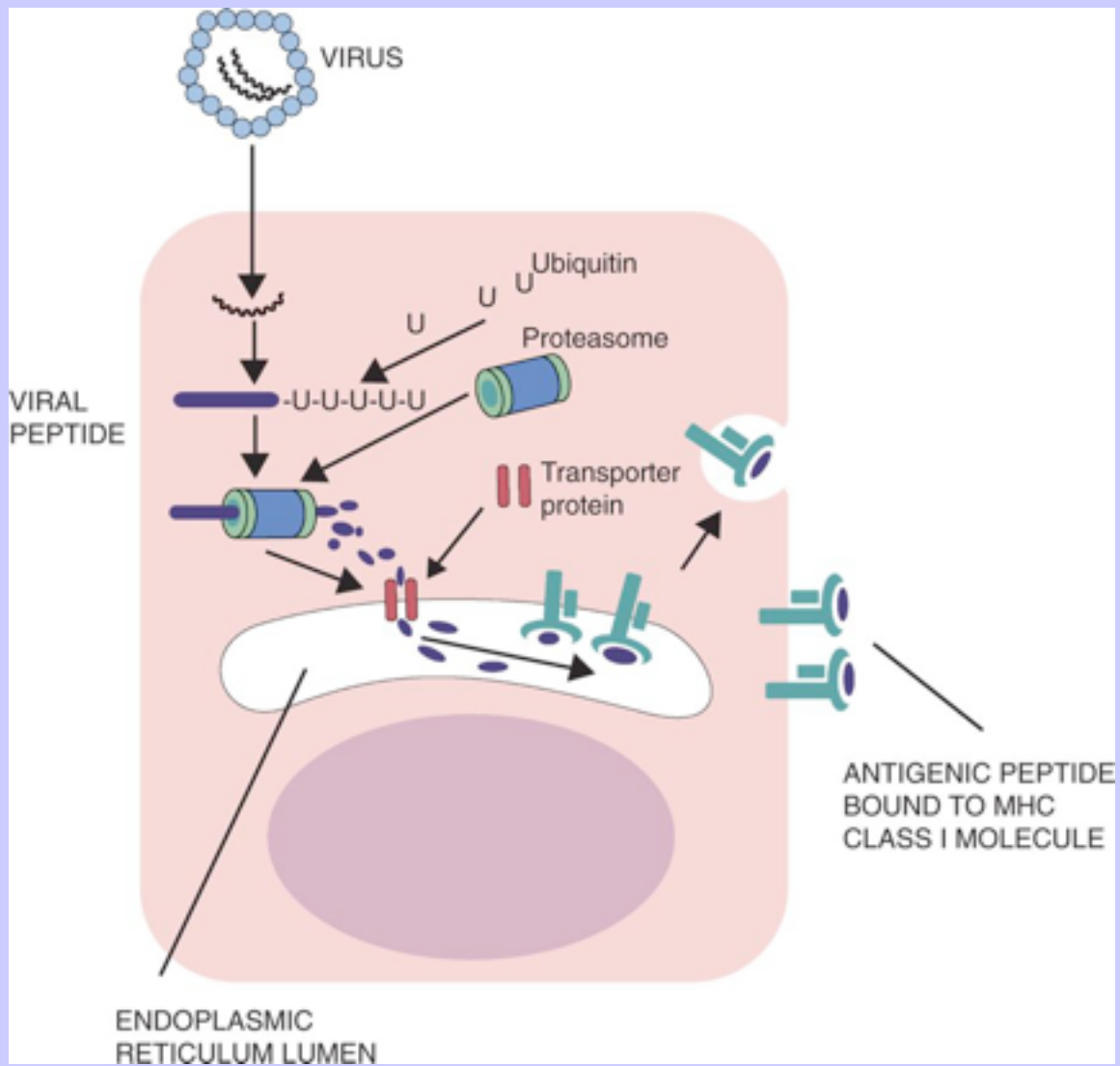
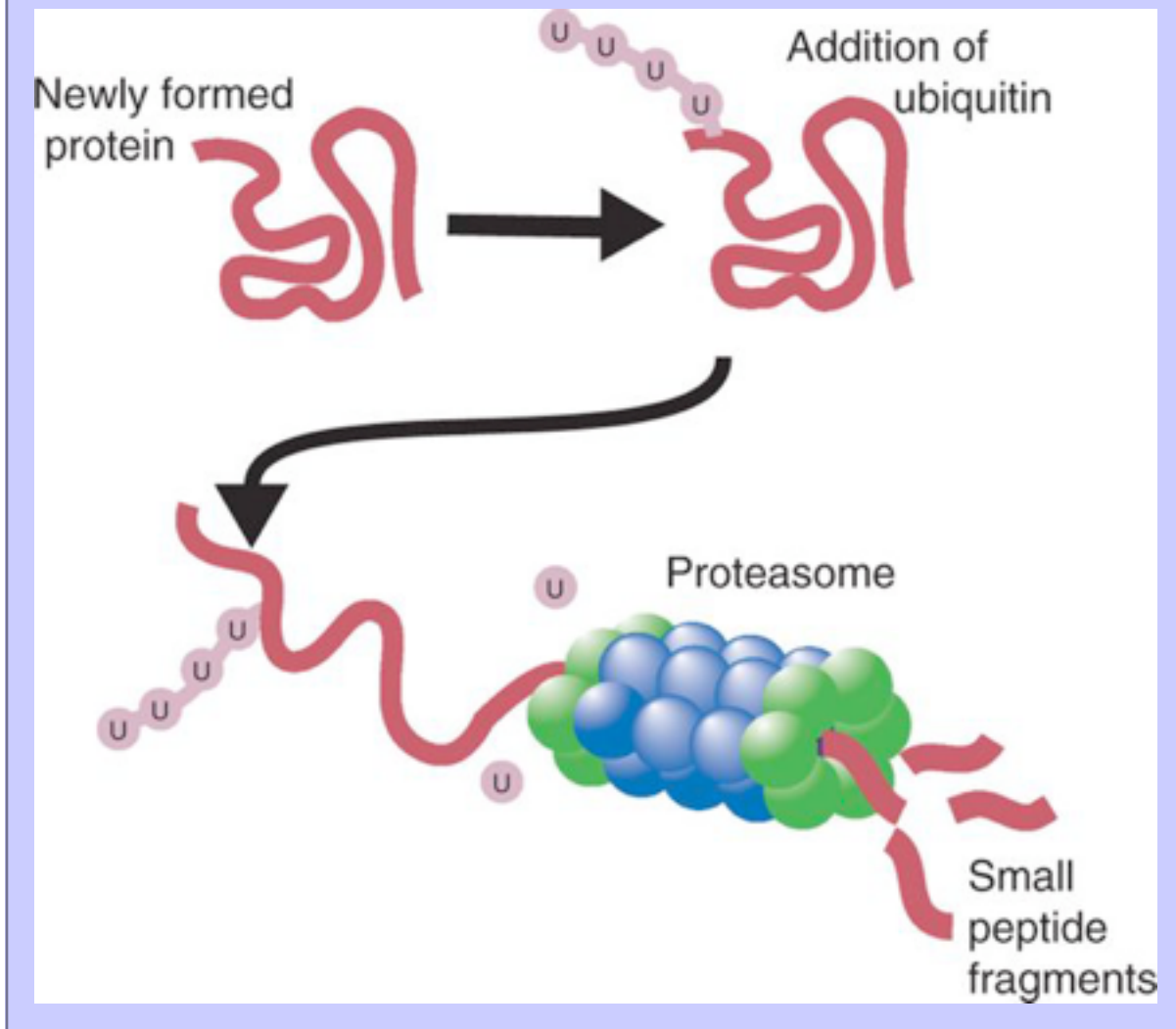


FIGURE 8-12 A diagram showing how the proteasome acts as a powerful protease. Ubiquitinated proteins are fed into its central channel, where they are cut into small peptides.



found in all eukaryote cells ([Figure 8-12](#)). Usually a chain of at least four ubiquitin molecules is added to the target protein—like beads on a string. Ubiquitinated proteins are marked for destruction. The ubiquitin chains are recognized by a large enzyme complex called a proteasome. Proteasomes are large, tubular molecular complexes consisting of an inner cylinder that contains the protease activity and two outer rings that regulate which proteins can enter the complex and be destroyed. Only ubiquitinated proteins can bind to the outer rings. The outer rings unfold the protein, release the ubiquitin for reuse, and thread the peptide chain into the inner cylinder, where it is broken into fragments between 8 and 15 amino acids long (like a meat grinder). The activity of proteasomes is regulated by cytokines such as IFN- γ and by caspases.

Most of these fragmented peptides are recycled into new proteins. For about one in a million molecules, however, the peptides are rescued from further breakdown by attachment to transporter proteins. Two transporter proteins have been identified—TAP1 and TAP2 (*TAP* standing for “transporter for antigen processing”). Both are encoded

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by genes located within the MHC. TAP1 and TAP2 select the peptides and transport them from the cytoplasm into endosomes. Here the peptides are attacked by an aminopeptidase, which shortens them one amino acid at a time until they are completely degraded, unless an intermediate, 9–amino acid peptide precisely fits the binding site on an empty MHC class I molecule. In this case, degradation stops and the MHC-peptide complexes are carried to the cell surface via the Golgi, where they are displayed for many hours.

A cell can express about 10^6 MHC-peptide complexes, and about 200 MHC class I molecules loaded with the same viral peptide are required to activate a cytotoxic T cell. Thus the MHC-peptide complexes provide fairly complete information on virtually all the proteins being made by a cell. Cytotoxic T cells can screen these peptides to determine whether any are “foreign” and bind to their TCRs.

8.6.1

Cross-Priming

Although it is usual for the two antigen-processing pathways to remain completely separate, under some circumstances exogenous antigens may enter the cytoplasm, join the endogenous antigen pathway, and be presented on MHC class I molecules. Thus in antigen-presenting cells such as macrophages and DCs, endocytosed viral antigen may not be degraded in lysosomes but transported from the phagosomes into the cytoplasm, where it is degraded by proteasomes and processed as an endogenous antigen. This antigen thus becomes associated with MHC class I molecules and is recognized by cytotoxic T cells. This may be important in immunity to viruses, since it implies that the antigens from dead virions may still be able to trigger a response by cytotoxic T cells (see [Chapter 16](#)). In general, however, cells that ingest an antigen through phagocytosis present it to T cells through MHC II–associated pathways.

8.7

HISTIOCYTOSIS AND HISTIOCYTOMAS

Domestic animals suffer from several diseases in which macrophage or DCs proliferate excessively. These are called histiocytomas or histiocytosis. Canine cutaneous histiocytoma is a benign epidermal neoplasm of Langerhans cell origin that usually regresses spontaneously. Histiocytomas are common in the dog but rare in goats and cattle. Langerhans cell histiocytosis is a reactive lesion whose trigger is unknown but may be an infectious agent. This condition is not premalignant, and it may occur in a cutaneous or systemic form. Both forms of Langerhans cell histiocytosis present with lesions in the skin or subcutis, but systemic histiocytosis also involves other tissues. Cutaneous histiocytosis shows no breed predilection, occurs in adult dogs between 3 and 9 years old, and is characterized by the development of nonpainful solitary or multiple nodules in the skin or subcutis. These lesions tend to occur on the head, neck, extremities, perineum, and scrotum. In contrast, systemic histiocytosis tends to occur in large breeds such as Bernese Mountain dogs, Rottweilers, Golden Retrievers, and Labradors. Its age of onset is between 4 and 7 years. The lesions develop in the skin, mucous membranes, eyes, nasal cavity, spleen, lung, liver, bone marrow, and spinal cord. Histologically these lesions are characterized by containing a mixture of cells. Phenotyping of both types of lesion shows the cells to be: CD1+, CD11c+, MHC II+, CD4+, and CD90+, a phenotype typical of Langerhans cells. The lesions also contain T cells and neutrophils and may be successfully treated with corticosteroids, cyclosporine, or leflunomide. As many as 30% of cutaneous cases and 10% of systemic cases spontaneously regress following infiltration by CD4+ T cells and the production of Th1 cytokines such as IL-2, TNF- α , and IFN- γ , as well as nitric oxide synthase 2, and subsequent recruitment of antitumor effector cells. Feline progressive histiocytosis is a skin disease presenting as solitary or multiple nonpruritic nodules on the feet, legs, and face. The histiocytes express CD1a, CD1c, CD18, and MHC class II molecules. Expression of E-cadherin, a characteristic of Langerhans cells, occurs in about 10% of cases. This is a slowly progressive disease that may, in its terminal stage, involve internal organs.

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9 CHAPTER 9 The Major Histocompatibility Complex

9.1 KEY POINTS

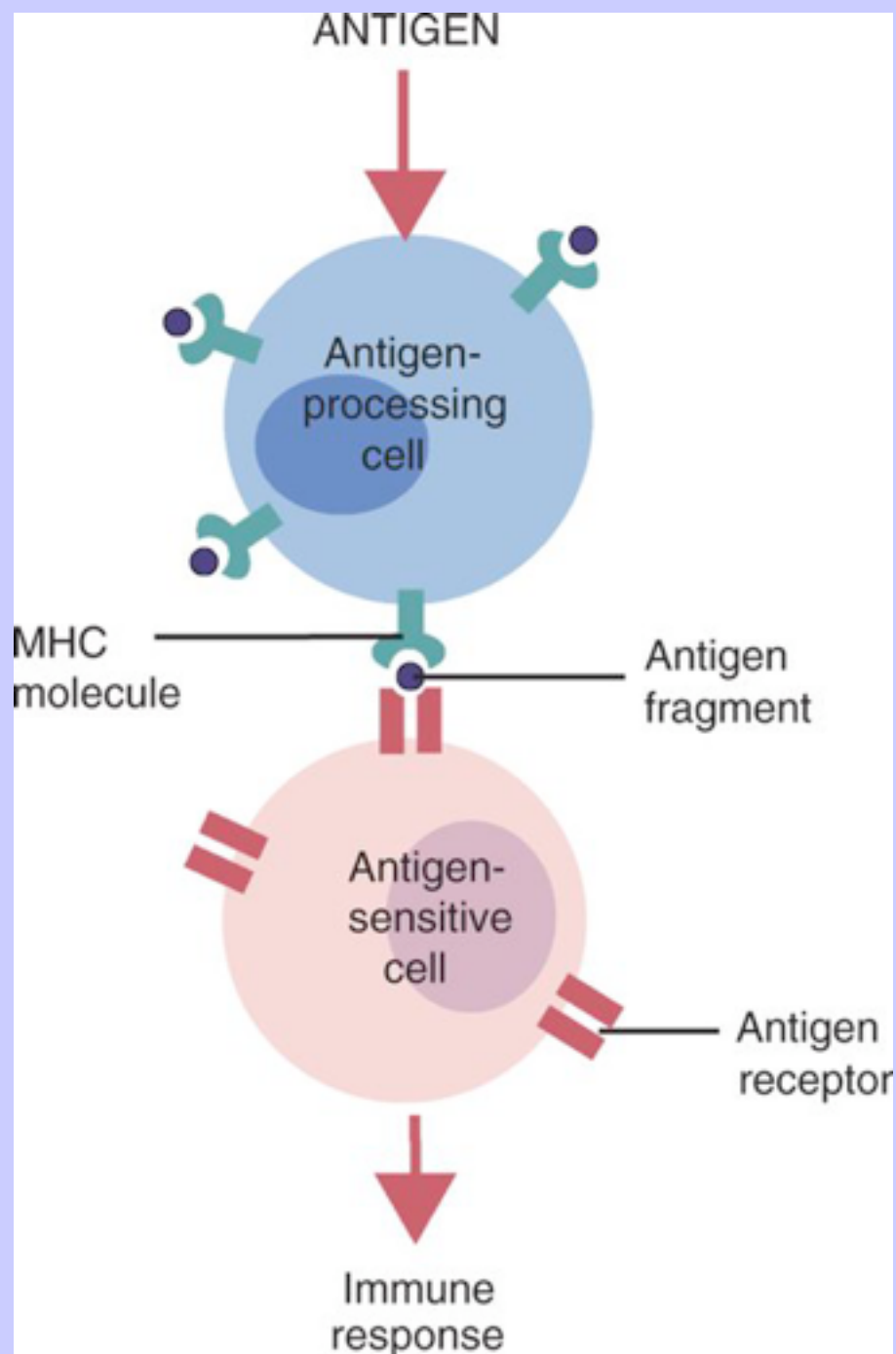
- Antigen-presenting cells use receptors called major histocompatibility complex (MHC) molecules to bind and present antigens.
- MHC molecules are encoded by genes located in the MHC.
- MHC molecules are highly polymorphic; that is, they show an enormous variety of inherited structural variations that permit each individual animal to respond to a different set of antigens.
- Class I MHC molecules are found on all nucleated cells. Their function is to present endogenous antigens to CD8+ T cells.
- Class II MHC molecules are found on the professional antigen-presenting cells, dendritic cells, macrophages, and B cells. Their function is to present exogenous antigens to CD4+ T cells.
- The class III region of the MHC contains a mixture of genes, some of which encode complement components.

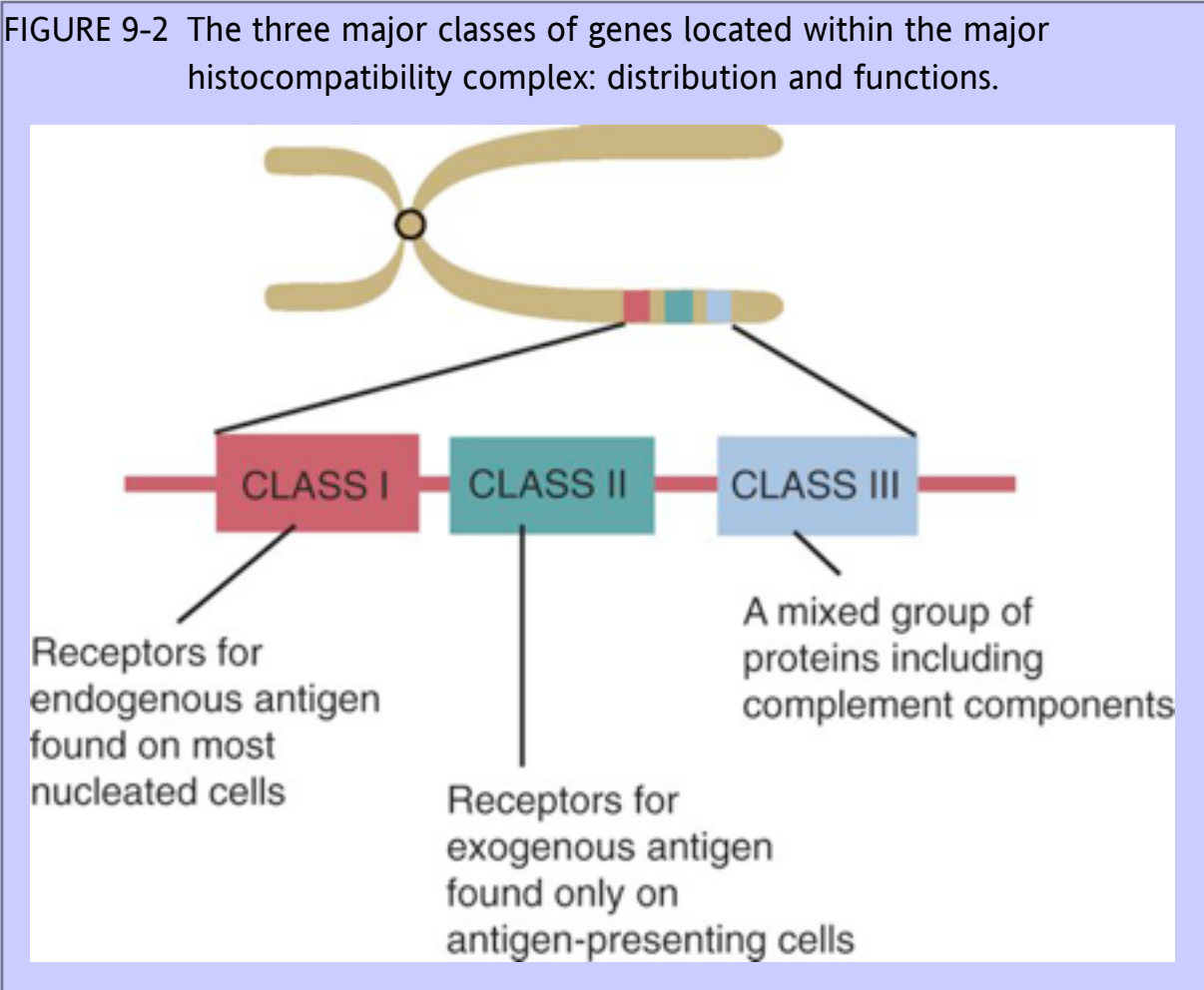
In order to trigger an acquired immune response, antigen molecules must be broken up inside cells and the antigen fragments generated must then be bound to appropriate antigen-presenting receptors ([Figure 9-1](#)). These antigen-presenting receptors are called histocompatibility molecules (or histocompatibility antigens). They are glycoproteins encoded by genes located in a gene cluster called the major histocompatibility complex (MHC). The receptors are therefore called MHC molecules. Antigen fragments can trigger an immune response only when they are bound to MHC molecules and these molecules can bind T cell antigen receptors. This requirement is called MHC restriction. Since the MHC molecules serve as specific antigen receptors, MHC genes determine which antigens can trigger acquired immunity. Thus the MHC can be considered an organized cluster of genes that control antigen presentation and so determine susceptibility to infectious or autoimmune diseases.

9.2 MAJOR HISTOCOMPATIBILITY COMPLEX

All vertebrates, from cartilaginous fish to mammals, possess histocompatibility molecules that are normally clustered within the MHC. Each MHC contains three classes of gene loci ([Figure 9-2](#)). Class I loci code for MHC molecules expressed on most nucleated cells. These class I loci can be classified as those that are highly polymorphic (class Ia loci) and those that show very little polymorphism (class Ib, Ic, or Id loci). *Polymorphism* refers to structural variations between

FIGURE 9-1 The key initial step in any immune response is the presentation of antigens by antigen-processing cells to antigen-sensitive cells. This step is performed by major histocompatibility complex (MHC) molecules located on the surface of antigen-processing cells.





proteins.) Class Id loci are located outside the MHC on a different chromosome. Class II loci, on the other hand, encode polymorphic MHC molecules found only on professional antigen-presenting cells (dendritic cells, macrophages, and B cells) ([Table 9-1](#)). MHC class III loci code for proteins with diverse functions, many of which are linked to innate immunity. For example, the class III loci contain genes coding for some complement proteins.

Table 9-1 A Comparison of MHC Class I and Class I Structure

	Class I	Class II
Loci include	Typically A, B, and C	DP, DQ, and DR
Distribution	Most nucleated cells	B cells, macrophages, and dendritic cells
Function	Present antigen to cytotoxic T cells	Present antigen to T helper cells
Result	T cell–mediated toxicity	T cell–mediated help

Although each MHC contains all three classes of loci, their number and arrangement vary. The collective name given to the proteins encoded by these MHC genes depends on the species. In humans these molecules are called human leukocyte antigens (HLAs); in dogs they are called DLA; in rabbits, RLA; in cattle (bovines), BoLA; in

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horses, ELA; in swine, SLA; and so forth. In some species, MHC molecules were identified as blood group antigens before their true function was recognized. In these cases, the nomenclature is anomalous. Thus in the mouse the MHC is called H-2 and in chickens it is called B. The complete set of alleles found within an animal's MHC is called its MHC haplotype.

9.3 MHC CLASS Ia MOLECULES

Class Ia molecules are expressed on most nucleated cells. In pigs, for example, class I molecules have been detected on lymphocytes, platelets, granulocytes, hepatocytes, kidney cells, and sperm. They are not usually found on mammalian red cells, gametes, neurons, or trophoblast cells. Some cells, such as myocardium and skeletal muscle, may express very few class Ia molecules.

9.3.1 Structure

Class Ia molecules consist of two linked glycoprotein chains. An α chain (45 kDa) is associated with a much smaller chain called β_2 -microglobulin (β_2 M) (12 kDa). The α chain is inserted in the cell membrane ([Figure 9-3](#)). It consists of five domains: three extracellular domains called α_1 , α_2 , and α_3 , each about 100 amino acids long; a transmembrane domain; and a cytoplasmic domain. The antigen-binding site on class Ia molecules is formed by the α_1 and α_2 domains. β_2 M consists of a single domain and serves to stabilize the structure.

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FIGURE 9-3 Diagram showing the structure of a class Ia major histocompatibility complex molecule on a cell membrane. Its antigen-binding site is formed by the folding of its α_1 and α_2 domains.

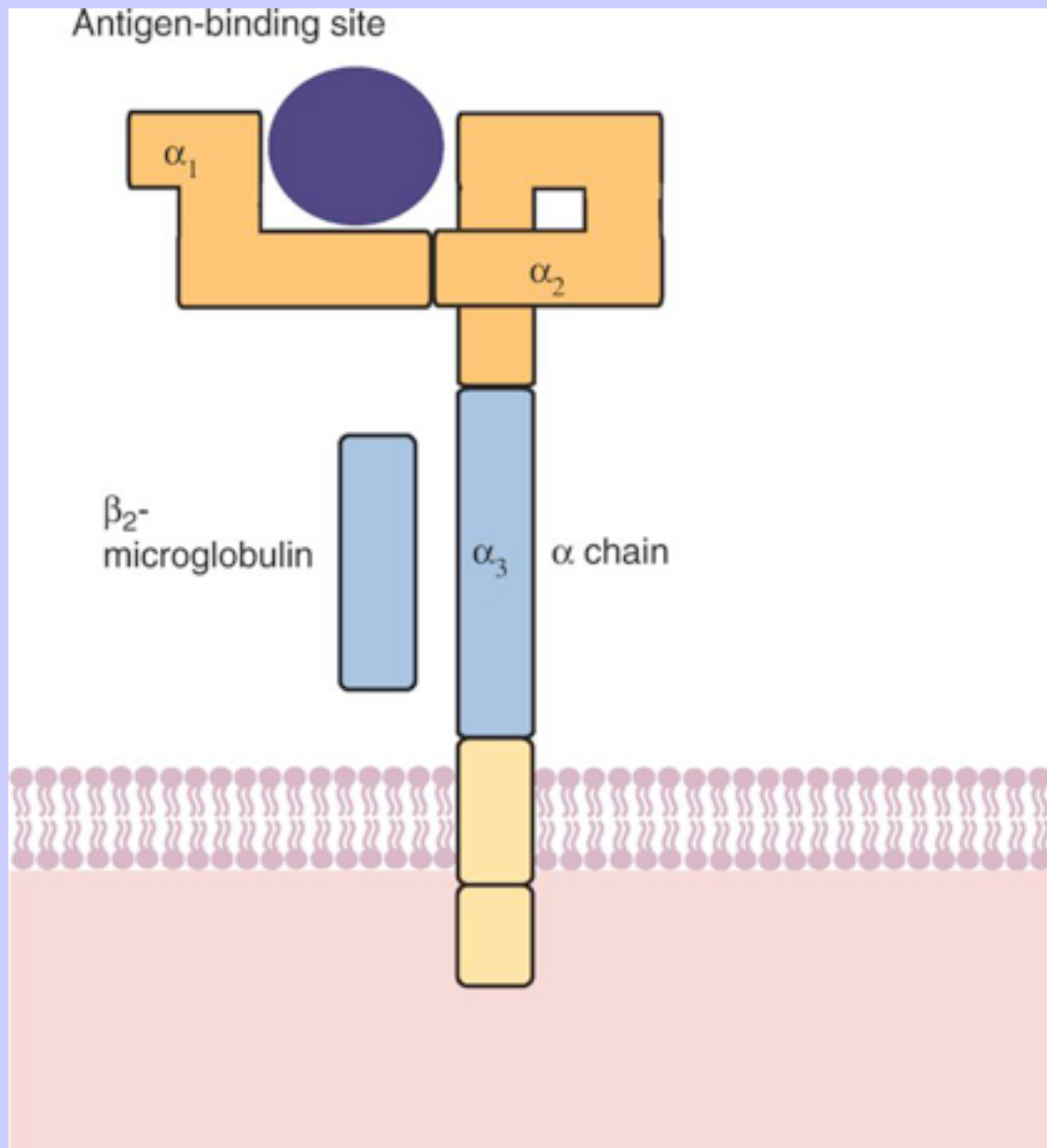


FIGURE 9-4 Arrangement of genes within the major histocompatibility complex (MHC) of the mouse, a typical mammalian MHC.

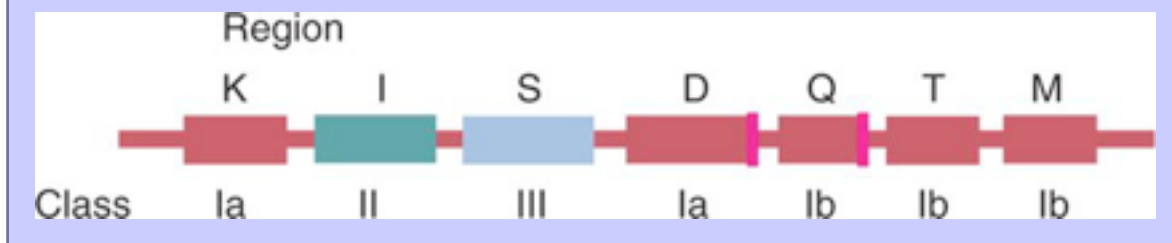
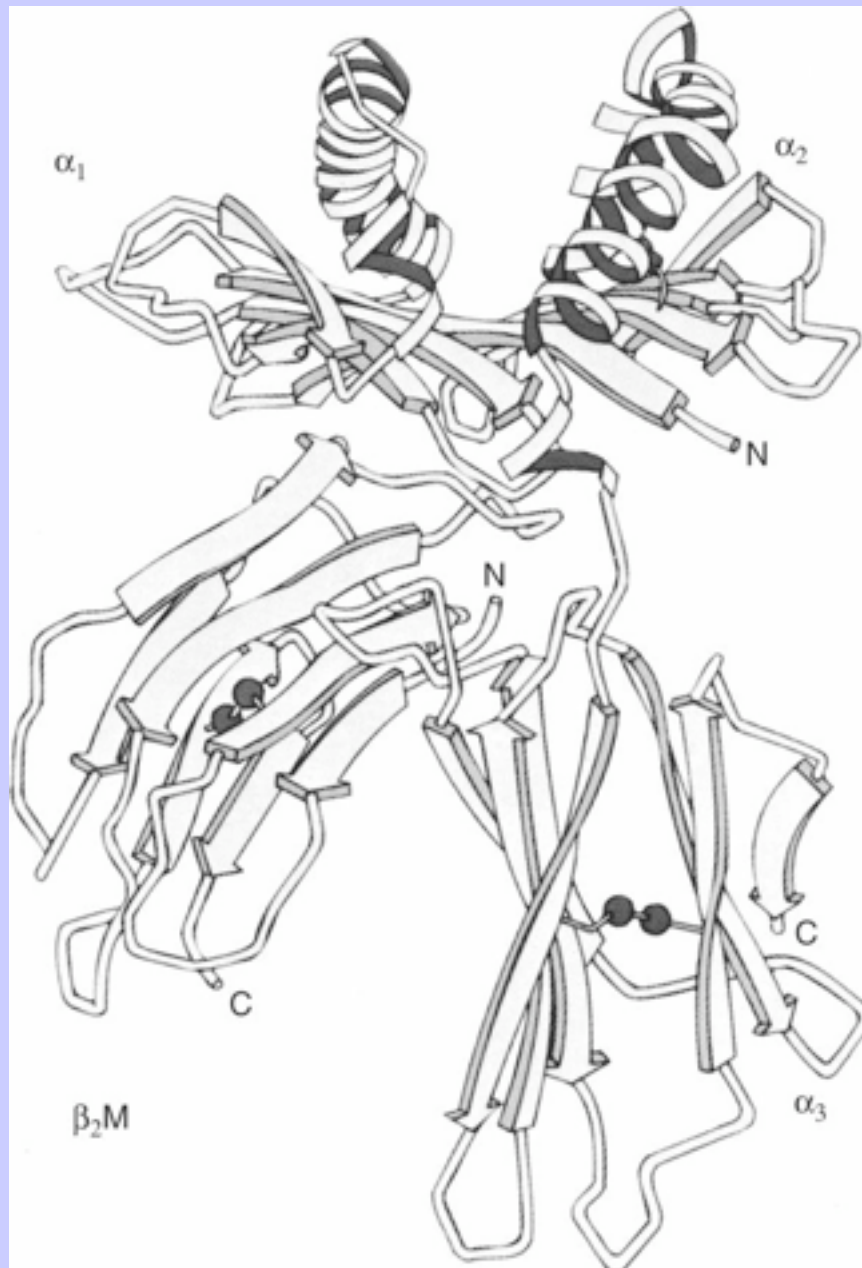


FIGURE 9-5 Schematic three-dimensional view of the complete structure of human leukocyte antigen-A2 derived by x-ray crystallography. The antigen-binding groove at the top is formed by the α_1 and α_2 domains, whereas the α_3 domain binds to the cell membrane. The β chain (b₂-microglobulin) has no direct role in antigen binding. (From Nature 320:506, 1987. Macmillan Magazines Ltd.)



9.3.2

Gene Arrangement

The size of the MHC class I region varies between mammals. Humans and rodents have the largest, and pigs have the smallest. The chicken class I region is much smaller than that in mammals (see [Chapter 37](#)). The MHC class I region has a common framework of non-MHC genes, and size differences are due to variations in the size and number of these interspersed genes.

The number of class Ia loci varies among mammals. For example, rats have more than 60; mice, about 30; humans, 20; cattle, 13 to 15; and pigs, 11. Not all these loci are functional. For example, in mice only two or three class I genes are expressed. The remainder are pseudogenes (defective genes that cannot be expressed). In humans the functional polymorphic loci are called *A*, *B*, and *C*. In mice they are called *K* and *D* (and in some strains, *L*) ([Figure 9-4](#)). In other species they are usually numbered.

9.3.3

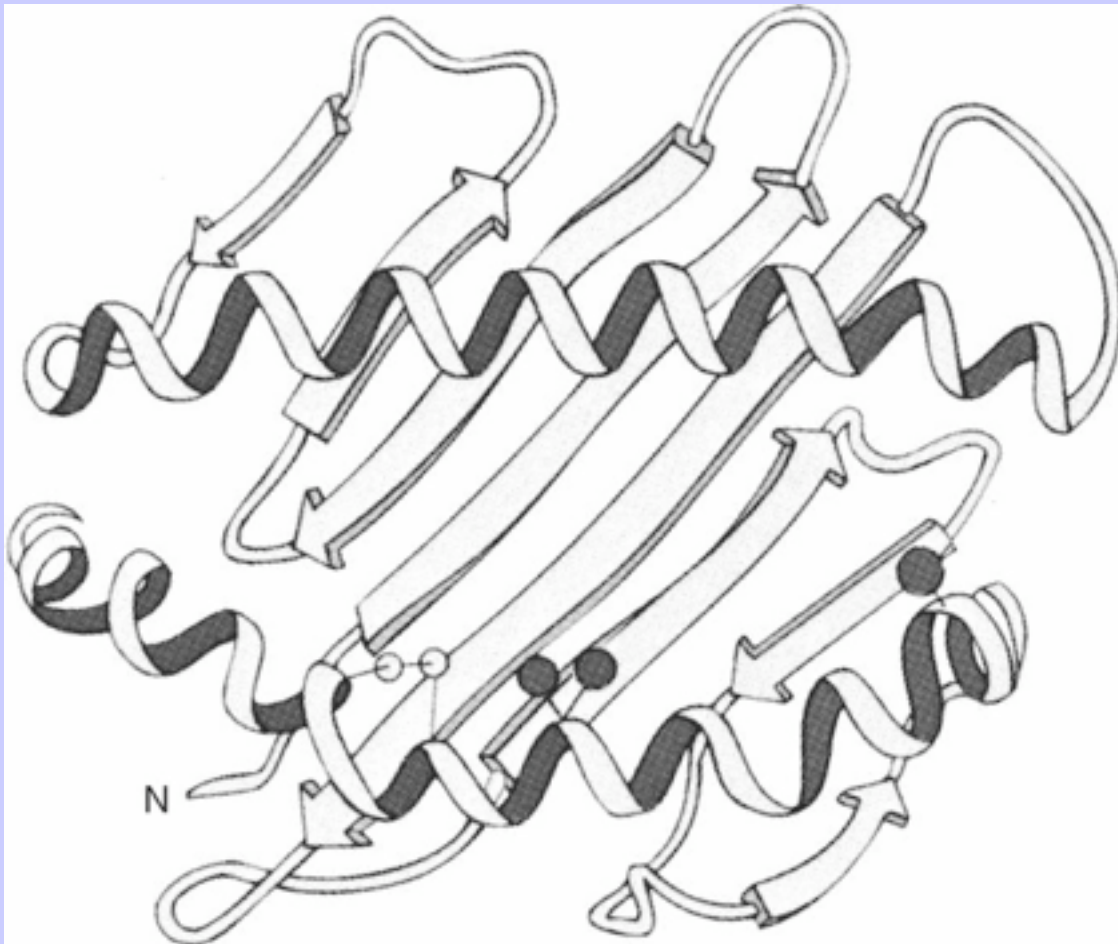
Polymorphism

Some of the class Ia loci encode very large numbers of alleles. These allelic differences cause variations in the amino acid sequences of the α_1 and α_2 domains. This variation is called polymorphism. Extreme polymorphism is restricted to three to four small regions within the α_1 and α_2 domains. In these variable regions, two or three different amino acids may occur at each position. The other domains of MHC class Ia molecules show little variation.

Analysis of MHC class Ia molecules has shown that their α_1 and α_2 domains fold together to form an open-ended groove ([Figure 9-5](#)). A flat β sheet forms the floor of this groove, and its walls are formed by two α helices ([Figure 9-6](#)). This groove binds antigenic peptides containing nine amino acids. The variable regions located along the walls of this groove determine its shape. The shape of the groove in turn determines which peptides can be bound and so trigger an immune response. The amino acid variability in the α_1 and α_2 domains results from variations in the gene sequence

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FIGURE 9-6 A view (from above) of the antigen-binding groove on a major histocompatibility complex class I molecule. The floor of the groove is formed by an extensive β -pleated sheet. The walls of the groove are formed by two parallel α helices. This structure is formed by the folding of the α_1 and α_2 domain of the α chain. (From Nature 320:506, 1987. Macmillan Magazines Ltd.)



between MHC alleles. The nucleotide variations are a result of point mutations, reciprocal recombination, and gene conversion. Point mutations are simply changes in individual nucleotides. Reciprocal recombination involves crossing over between two chromosomes. In gene conversion, small blocks of DNA are exchanged between different class I genes in a nonreciprocal fashion. The donated DNA blocks may come from nonpolymorphic class I genes, from nonfunctional pseudogenes, or from other polymorphic class I genes. Class I MHC genes have the highest mutation rate of any germ-line genes yet studied (10^{-3} mutations per gene per generation in mice). This high mutation rate implies that there must be significant advantages to be gained by having very polymorphic MHC genes.

9.4 NONPOLYMORPHIC MHC CLASS I MOLECULES

Mammalian cells also express many nonpolymorphic class I molecules on their surface. Some are encoded by genes within the MHC, others by genes on other chromosomes. They are classified according to their evolutionary origin.

Class Ib molecules show reduced expression and tissue distribution compared with class Ia molecules but are part of the MHC complex. They have limited polymorphism and probably originated from class Ia precursors by gene duplication. For example, the class Ib gene loci in mice are found in three loci called Q, T, and M (see [Figure 9-4](#)). They code for proteins on the surface of regulatory and immature lymphocytes and on hematopoietic cells. They consist of a membrane-bound α chain (44 kDa) associated with β_2 -microglobulin. Their overall shape is similar to that of the class Ia molecules, and they have an antigen-binding groove. Since they are not polymorphic, MHC class Ib molecules bind a limited range of ligands. They are receptors for commonly encountered microbial pathogen-associated molecular patterns (PAMPs). For example, the mouse class Ib molecule, M3, binds to peptides that contain N-formylmethionine at their amino terminus.

Class Ic genes are low-polymorphic molecules that are found within the MHC but probably originated before the radiation of the placental mammals. This group includes MICA and MICB, specialized molecules that are involved in communicating with T cells and natural killer (NK) cells but that do not bind to peptides (see [Chapter 30](#)).

Class Id genes are nonpolymorphic class I-related genes not located on the MHC chromosome. Since they bind PAMPs, many of these molecules contribute to innate immunity. For example, CD1 molecules bind bacterial lipids including mycolic acids and glycolipids from mycobacteria. FcRn is a class Id MHC molecule that serves as an antibody (Fc) receptor on epithelial cells. It is expressed on mammary gland epithelium and on the enterocytes of newborn mammals (see [Chapter 19](#)). FcRn binds antibodies ingested in mother's milk and transfers them to the bloodstream.

9.5 MHC CLASS II MOLECULES

Mammals differ in their expression of MHC class II molecules. In rodents, they are expressed constitutively on the professional antigen-presenting cells (dendritic cells, macrophages, and B cells) and can be induced on T cells, keratinocytes, and vascular endothelial cells. Resting mouse T cells do not express MHC class II molecules, but in pigs, dogs, cats, mink, and horses the MHC class II products are constitutively expressed on nearly all resting adult T cells. In cattle, most MHC class II molecules are expressed only on B cells and activated T cells. In pigs, resting T cells express MHC class II molecules at about the same level as macrophages. They are also present on boar sperm. In humans and pigs MHC class II molecules are expressed on renal vascular endothelium and glomeruli—a fact of significance in kidney graft rejection. The expression of class II molecules is enhanced in rapidly dividing cells and in cells treated with interferon- γ (see [Chapter 29](#)).

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9.5.1 Structure

MHC class II molecules consist of two protein chains called α (31 to 34 kDa) and β (25 to 29 kDa). Each chain has two extracellular domains, a connecting peptide, a transmembrane domain, and a cytoplasmic domain ([Figure 9-7](#)). A third chain, called the Ii or γ chain, is associated with intracellular class II molecules and was discussed in [Chapter 8](#).

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9.5.2 Gene Arrangement

A “complete” class II region contains three paired loci. In primates, these are DPA and DPB, DQA and DQB, and DRA and DRB. (The genes for the α chains are designated A, and the genes for the β chains are called B.) Some, but not all, of these genes are polymorphic. In humans there may also be some nonpolymorphic loci such as DM and DO. The DO and DM loci code for molecules whose function is to regulate the loading of antigen fragments into the groove. However, not all mammals possess a “complete set.” Not all loci contain genes for both chains, and some contain many pseudogenes. These pseudogenes serve as donors of DNA that can be used to generate additional class II polymorphism by gene conversion.

9.5.3 Polymorphism

Class II proteins have an antigen-binding groove formed by their α_1 and β_1 domains. Its walls are formed by two parallel α helices, and its floor consists of a β sheet. Polymorphism results from variations in the amino acids forming the sides of the groove. These variations are generated in the same way as class Ia molecules. Other molecules encoded by genes in the class II region are also involved in antigen-processing. These include the transporter proteins transporter for antigen processing-1 (TAP1) and TAP2 and some proteasome components (see [Chapter 8](#)).

9.6 MHC CLASS III MOLECULES

The remaining genes located within the MHC are classified as class III genes ([Figure 9-8](#)). They code for proteins with many different functions. Some are important in the defense of the body, including the genes for the complement components C4, factor B, and C2 (see [Chapter 5](#)). They also include genes that encode the enzyme 21-hydroxylase involved in steroid synthesis, cytochrome P450, tumor necrosis factor (TNF- α), several lymphotoxins, some NK cell receptors, and several heat shock proteins.

9.7 MHC OF DOMESTIC ANIMALS

Every mammal studied has an MHC containing class I, class II, and class III genes. When the MHCs of different mammals are compared, some regions are well conserved whereas others are highly diverse. Likewise,

FIGURE 9-7 Diagram showing the structure of a major histocompatibility complex class II antigen located on a cell surface. Note that the antigen-binding site is formed by both peptide chains.

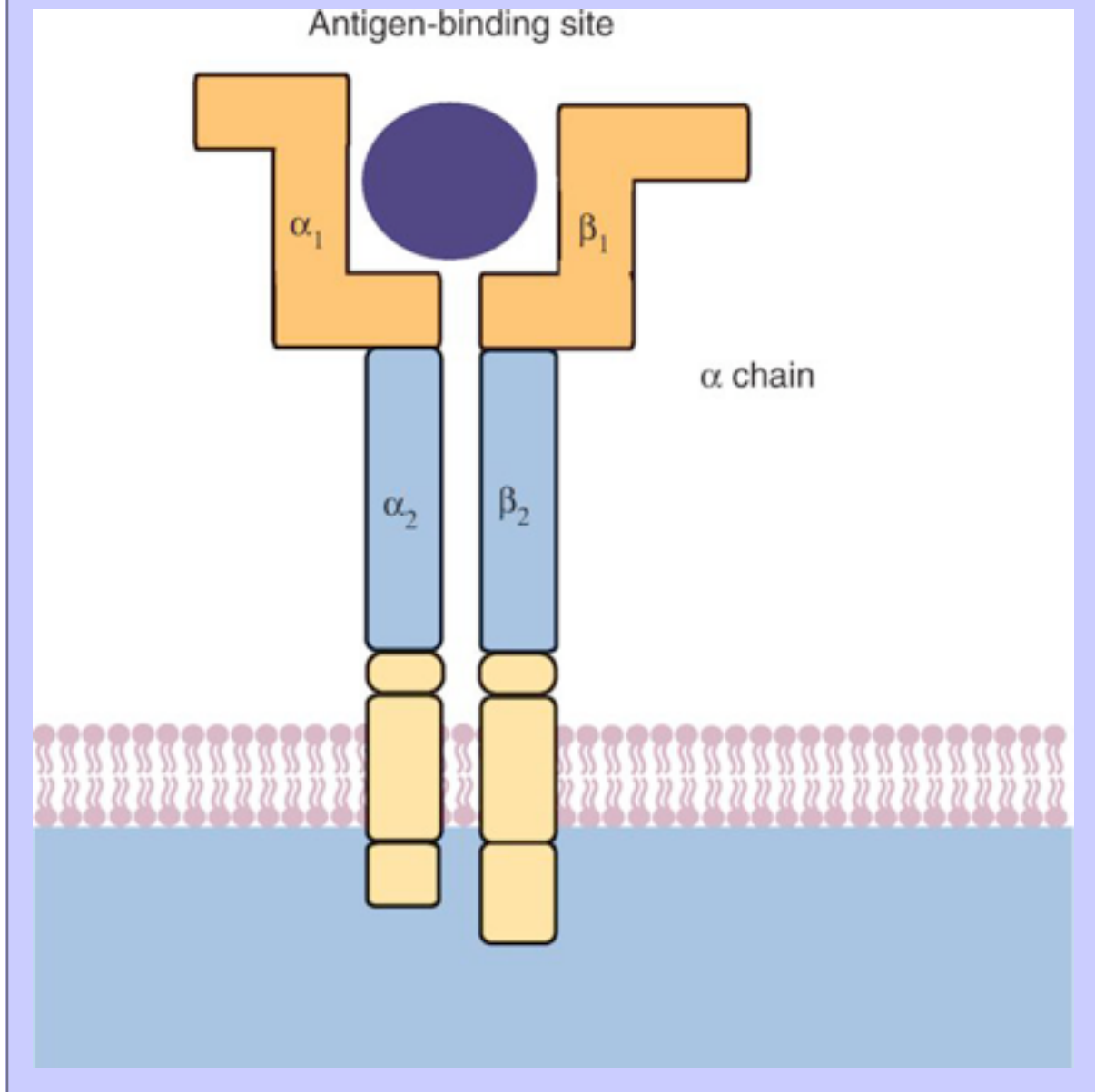
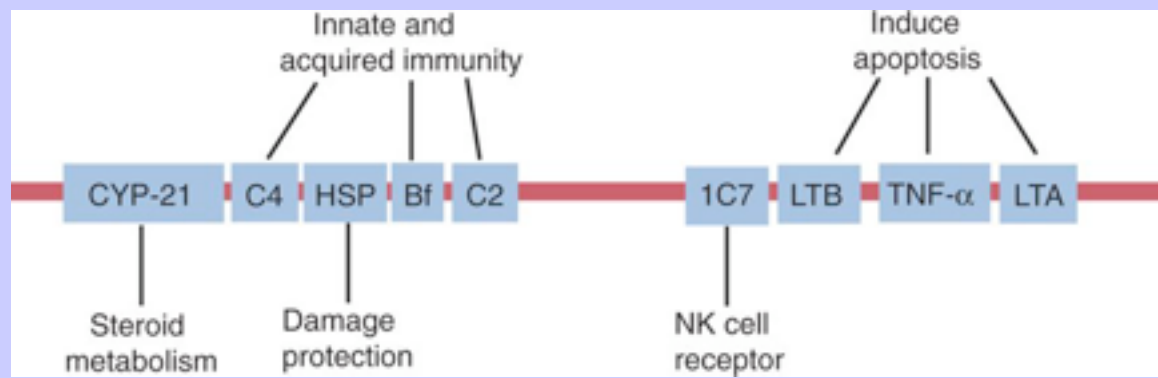
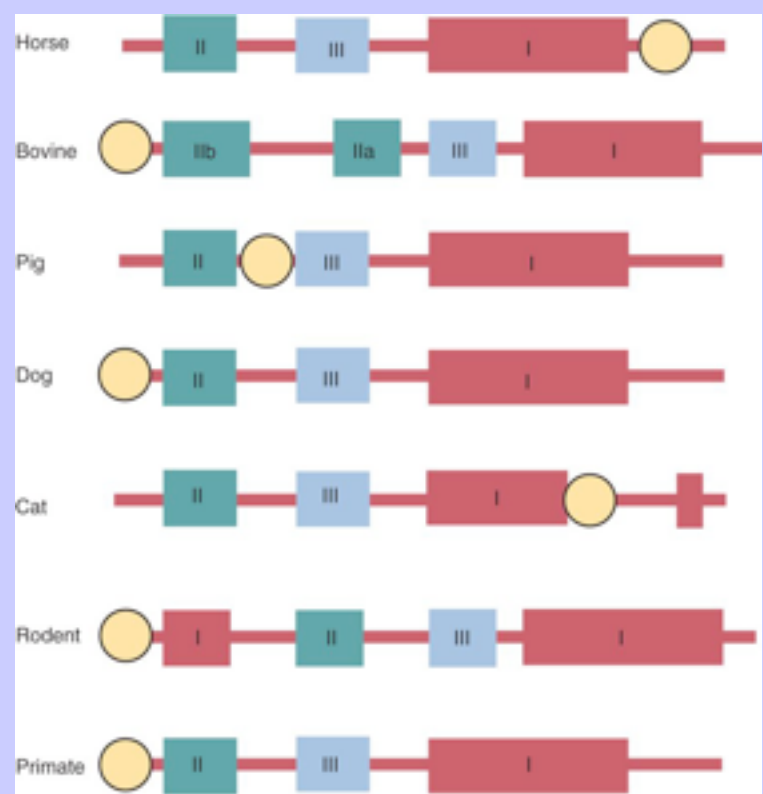


FIGURE 9-8 The arrangement of selected genes within the major histocompatibility complex class III region. These genes were selected because their functions are related to innate and acquired immunity. There are many other genes in this region that have no apparent role in immunity.



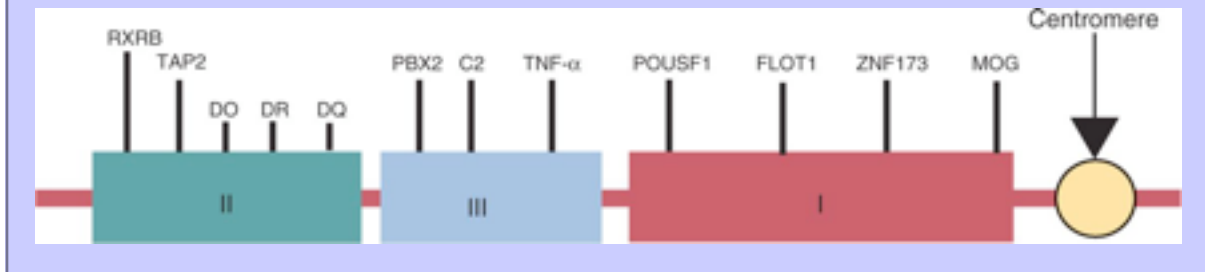
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FIGURE 9-9 Arrangement of gene regions within the major histocompatibility complex in different species of domestic mammals.



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FIGURE 9-10 Schematic diagram showing the overall arrangement of the equine major histocompatibility complex (equine leukocyte antigen). (Courtesy Drs. A.L. Gustafson and L. Skow.)



the precise arrangement and number of loci varies among species ([Figure 9-9](#)). In general the class II and class III genes are orthologous; that is, they are clearly derived from a single ancestor and have not usually been subjected to major rearrangements during evolution. (Ruminant class II genes are an exception.) Class I genes, in contrast, have been reorganized so many different times by deletion and duplication that their amino acid sequences differ widely and it is very difficult to compare them in different species. They are said to be paralogous.

9.7.1

Horse

In the horse, the ELA complex is of conventional structure but its sequence is reversed in relation to the centromere ([Figure 9-10](#)). It is located on chromosome 20. Unlike other mammals, the equine DRA locus is polymorphic with at least 11 alleles. Horses possess at least seven expressed class I genes and eight class I pseudogenes.

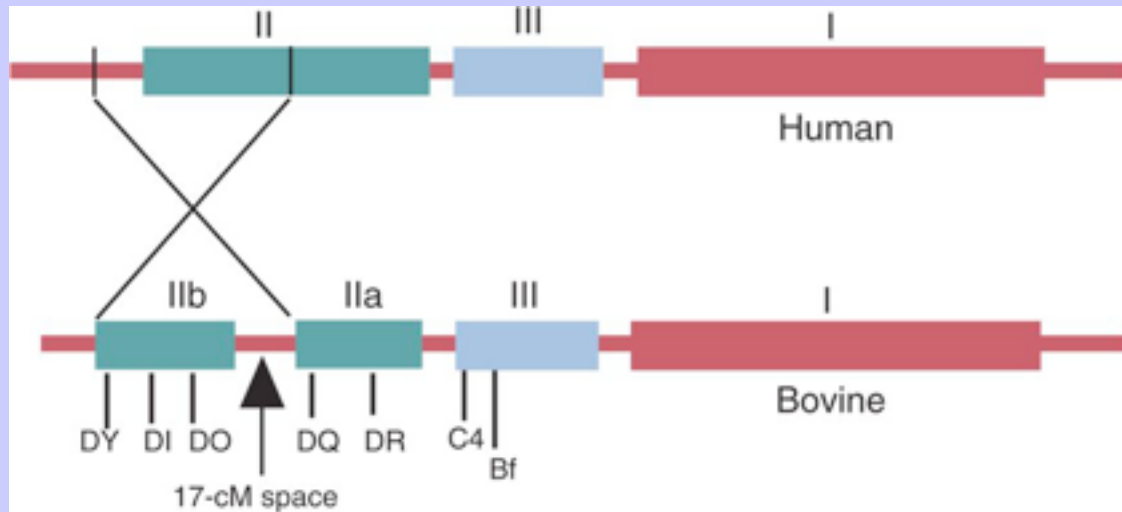
9.7.2

Cattle

In cattle, the MHC is structurally unique in that inversion of a large chromosome segment has moved several class II genes close to the centromere of bovine chromosome 23. As a result, the BoLA class II region is divided into two subregions called IIa and IIb. The class IIb genes are separated from the class IIa genes by a “gap” of 17 centimorgans (cM) ([Figure 9-11](#)).

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FIGURE 9-11 Arrangement of genes within the human and bovine major histocompatibility complex. The only major difference is the presence of a large inversion at the 5' end of the bovine class II region that leads to the development of a large gap within the bovine class II region.



Cattle express only two class II proteins, DQ and DR, although in many cattle haplotypes the DQ locus is duplicated. Because of this duplication, additional MHC diversity can be generated by interhaplotype pairing: that is, DQA and DQB gene products from different chromosomes can be paired. The DRA chain in cattle is not polymorphic, and polymorphism in the DRB chains is the only source of DR diversity. Both DQA and DQB are polymorphic. Cattle, sheep, and goats possess ruminant-specific class II genes (DYA, DYB, and DI) and lack a DP locus.

Cattle have six class Ia loci but only one, or combinations of two or three, are expressed in different haplotypes (e.g., 1,2,4 or 3,5 or 2,3). Some combinations are more common than others. Thus there are three common, equally polymorphic genes that are expressed only in certain combinations. One of these (gene 2) is expressed on nearly all haplotypes. Genes 1 and 3 are never expressed together. Genes 4, 5, and 6 are expressed in a limited number of haplotypes. There is also some inter-locus recombination resulting in the production of “hybrid genes.”

Mammals use two distinct strategies for maintaining high levels of MHC class I polymorphism. In mice and humans the strategy is to use a small number of highly polymorphic genes. In primates and rats, however, polymorphism is generated by varying the number and combinations of genes transcribed. Cattle use both strategies by employing various combinations of six or more classical class I genes, but three of these are also highly polymorphic.

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9.7.3

Pig

The SLA complex is located on chromosome 7 and is divided by the centromere. The class I and III regions are therefore located on the short arm, and the class II region is located on the long arm of this chromosome. The pig MHC is the smallest yet found among the mammals since it measures only about 2 Mb. Pigs have 11 class Ia genes, only three of which are functional. Others may be transcribed, and three are clearly pseudogenes. As in cattle, the number of expressed class I genes varies between haplotypes. Of the pig class II genes, some code for *DR* and *DQ* heterodimers but there is no *DP* product.

9.7.4

Dog

The DLA complex is located on chromosome 12. There are four transcribed class I genes (DLA-12, -79, -64, and -88), but only one, DLA-88, is polymorphic. DLA-DRA, DRB, DQA, and DQB class II loci have been identified, and many are highly polymorphic. For example, to date, 62 DRB1, 21 DQA1, and 48 DQB1 alleles have been identified. Some class II haplotypes appear to be characteristic of certain breeds, but variations between breeds are great. This high interbreed but low intrabreed variation likely accounts for the differences in breed susceptibility to infectious and auto-immune diseases.

9.7.5

Cat

The cat MHC is intermediate in size between mouse and human and is located on chromosome B2. Its class I region appears to contain only one functional polymorphic locus. Its class II region has no functional *DP* genes, and its *DQ* region has been deleted (a feature seen only in the cat). In compensation, it has a highly polymorphic *DR* locus with at least two *DRB* genes and 24 alleles and three *DRA* genes. The FeLA class I region is divided into two regions by the centromere.

9.7.6

Humans

The human MHC, known as HLA, contains three class Ia loci—A, B, and C—and at least three functional class Ib loci—E, F, and G. Most Old World primates possess all of these except for C, which is found only in humans, gorillas, and chimpanzees, and G, which is found only in humans. In orangutans and rhesus monkeys, the A and B loci are duplicated. In contrast, the New World primates, such as cotton-top tamarins, have MHC class I genes most closely related to HLA-G. They do not possess any genes related to HLA-A, -B, or -C. In view of the lack of class I MHC diversity in cotton-top tamarins, it is perhaps not surprising that they are susceptible to fatal infection with viruses that are not fatal in humans.

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9.8

MHC MOLECULES AND DISEASE

Since the function of MHC molecules is to present antigens to the cells of the immune system, MHC genes regulate immune responses. A foreign molecule that cannot bind to the groove of at least one MHC molecule will not stimulate acquired immunity ([Figure 9-12](#)). Thus MHC alleles determine susceptibility to diseases in which immune responses have a significant role. These include not only infections but also autoimmune diseases.

Because class Ia and class II MHC molecules are polymorphic, each MHC allele will bind a different set of antigenic peptides. The more variety in an animal's MHC, the more antigens it can respond to. Thus an MHC

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heterozygous animal will express more alleles and can bind a greater variety of antigenic peptides than can a homozygous animal ([Figure 9-13](#)).

MHC polymorphism is maintained in populations by a process called overdominant selection or heterozygote advantage. Simply put, MHC heterozygotes are at an advantage because they can respond to more antigens and so are best fitted to survive infectious diseases. The antigen-binding site of an MHC class Ia or II molecule is also very nonspecific (or degenerate), and it has been estimated that an average MHC molecule can bind about 2500 different peptides. This is because the MHC groove binds tightly to the peptide backbone rather than to the amino acid side chains. Nevertheless, structural constraints limit the efficiency of binding of each allele. As a result, it is likely that only one or two peptides from an average antigenic protein can bind to any given MHC molecule. The ability of MHC molecules to bind antigens must be a limiting factor in generating acquired immunity and resistance to infectious agents. Increasing the diversity of MHC molecules will clearly increase the diversity of peptides that can be bound and so increase resistance to infections. Because most individuals are MHC heterozygotes, each individual normally expresses at most six different class Ia molecules. (In humans, for example, two each are coded for by the HLA-A, -B, and -C loci.) The number of expressed MHC molecules is not larger, because that would increase the possibility that the MHC molecules could bind and present more “self” antigens. This would require the elimination of many more self-reactive T cells during

FIGURE 9-12 Major histocompatibility complex (MHC) molecules regulate the immune response. Only molecules that can bind in the groove of an MHC molecule will trigger an immune response. This is called MHC restriction. Thus the MHC genes that code for these molecules also regulate immune responsiveness.

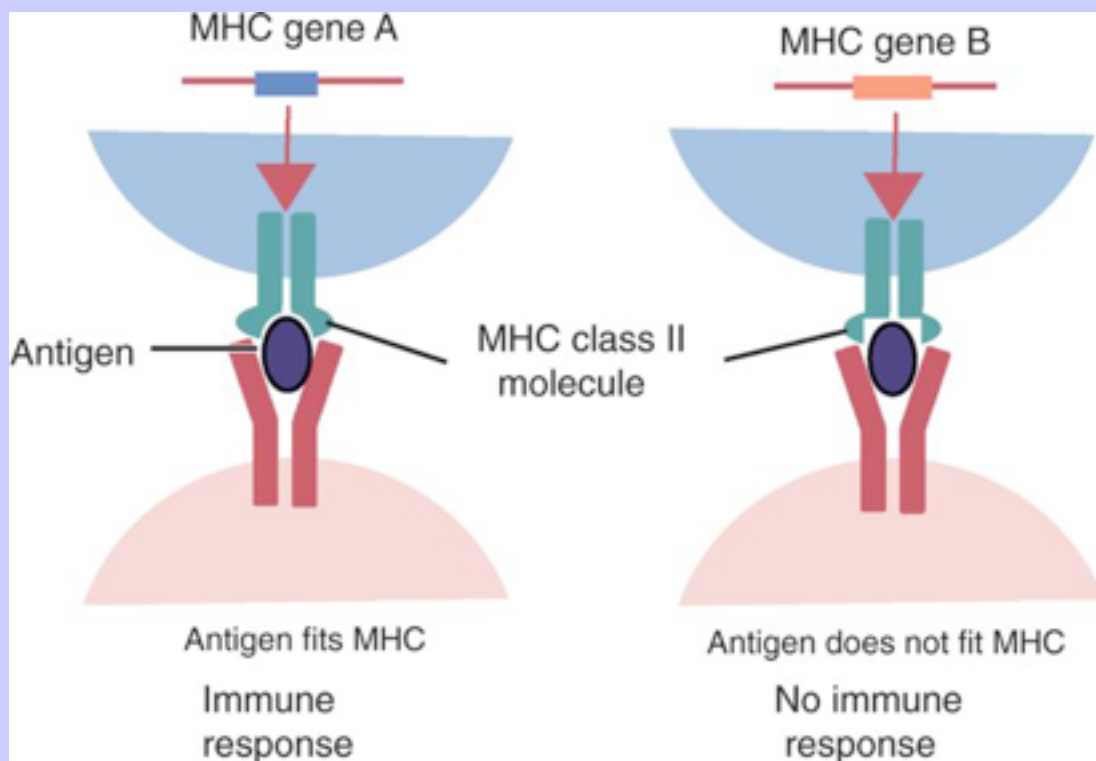
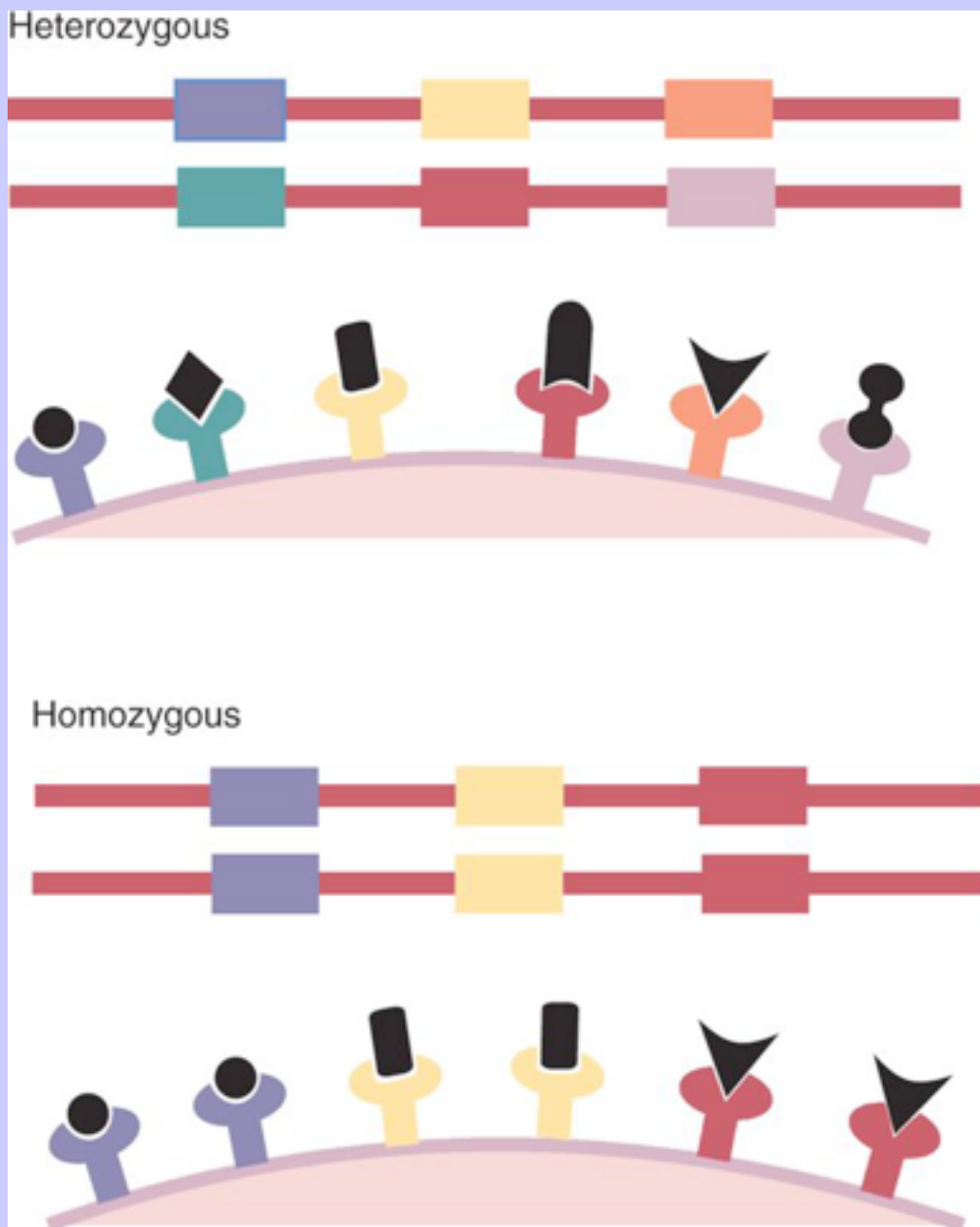


FIGURE 9-13 Heterozygous animals with two types of major histocompatibility complex (MHC) molecule coded for at each locus express six different antigen-presenting molecules on the cell surface. Therefore they generate a more diverse and effective immune response than homozygous animals with only one MHC molecule coded for at each locus. An example of heterozygote advantage.



development (see [Chapter 17](#)). Thus six different MHC class Ia molecules represent a reasonable compromise between maximizing recognition of foreign antigens and minimizing recognition of self-antigens ([Figure 9-14](#)).

Some MHC class Ia loci contain highly polymorphic genes that code for very large numbers of alleles. For example, the H-2K locus in the mouse codes for more than 100 alleles. Since there can never be more than two alleles/locus in an individual, it appears that this number of alleles is needed to maximize polymorphism in a mouse population. One possible reason for this is to protect the population as a whole from disease. Because of MHC polymorphism, most individuals in a population have a unique set of class Ia molecules and each individual can respond to a unique set of antigens. When a new infectious disease strikes a population, it is likely that at least some individuals will have MHC molecules that can bind the new microbial antigens and trigger immunity. Those that can respond will mount an immune response and live. Those that cannot respond will die.

When large populations of humans or mice are examined, no single MHC haplotype is present at a very high frequency. In other words, no single MHC haplotype confers major advantages on an individual animal. This reflects the futility of the host attempting to match invading organisms in antigenic variability. A microbe will always be able to mutate and evade the immune response faster than a mammalian population can develop resistance. Any changes in an MHC allele, while they may increase resistance to one organism, may at the same time decrease resistance to another. It is more advantageous therefore for the members of a population to possess multiple uniquely different MHC alleles so that any pathogen spreading through a population will have to adapt anew to each individual.

Highly adaptable social animals, such as humans or mice, with large populations through which disease can spread rapidly, usually show extensive MHC polymorphism ([Figure 9-15](#)). In contrast, low-density solitary species such as the marine mammals (whales and elephant seals), moose, or Asiatic lions, have much less polymorphism. It is also of interest to note the case of the cheetah, which has minimal polymorphism as a result of recent population bottlenecks. Because of this lack of MHC diversity, cheetahs will accept allografts from other, unrelated cheetahs. Likewise an infectious disease such as feline infectious peritonitis causes 60% mortality in cheetahs compared with 1% to 2% mortality in domestic cats and has the potential to cause the cheetah's extinction.

There are many examples of relationships between MHC haplotype and its resistance to infectious disease. For example, in cattle there is an association between possession of certain BoLA alleles and resistance to bovine leukosis, squamous cell eye carcinoma, and trypanosomiasis; responsiveness to foot-and-mouth disease virus; and susceptibility to the tick *Boophilus microplus*.

Cows with BoLA-Aw8 are more likely to be seropositive for leukosis, a disease caused by bovine leukemia virus (BLV). Resistance is associated with possession of BoLA-Aw7, and susceptibility is associated with possession of BoLA-Aw12. B cell proliferation and expression of BLV-induced B cell tumors are also controlled by BoLA. BoLA-Aw14 seems to influence age at seroconversion, whereas BoLA-Aw12 seems to be associated with susceptibility to B cell proliferation. However, these associations with the BoLA-A locus are relatively weak compared with the association between susceptibility and certain BoLA-DRB alleles such as DRB3. BoLA-DRB3 polymorphism influences resistance or susceptibility to BLV. This resistance is associated with the presence of two amino acids, glutamic acid-arginine, in the antigen-binding site of DRB3.

FIGURE 9-14 The optimal number of major histocompatibility complex (*MHC*) molecules is a balance between the need to respond to as many different microbial antigens as possible and the need to avoid autoimmune responses. Computer modeling suggests that the optimal number of *MHC* molecules is six.

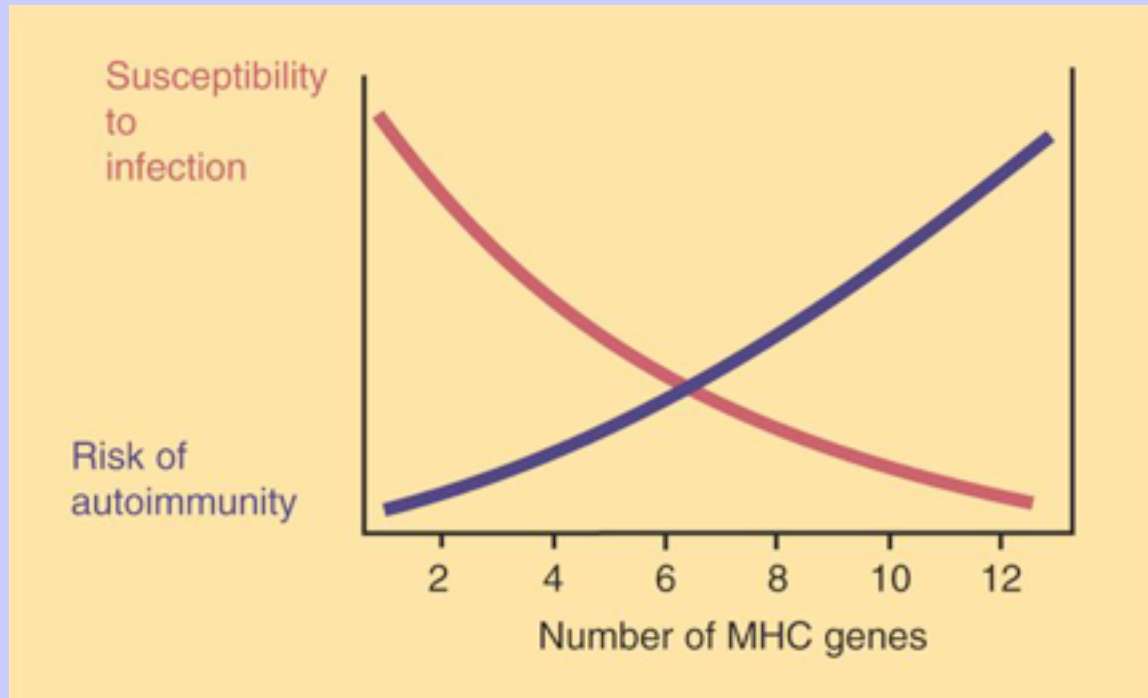


FIGURE 9-15 An example of how major histocompatibility complex (MHC) polymorphism can generate an enormous number of different MHC haplotypes. The numbers above each locus are the number of identified alleles in the human MHC as of January 2007. The number of different combinations can be determined by multiplying all of them together. Thus there are 13×10^9 class II combinations, 12×10^7 class I combinations, and 1.7×10^{18} total possible combinations, more than sufficient to give every human a unique haplotype.



at positions 70 and 71, whereas val-asp-thr-tyr at positions 75 to 78 is associated with susceptibility.

BoLA-A*16 is associated with resistance to mastitis. BoLA-A*6 and BoLA-A*16 are associated with high, and BoLA-A*2 with low, antibody responses to human serum albumin. Disease associations are also seen with class II alleles. Thus possession of BoLA-DRB3.2*23 is associated with an increased incidence of severe coliform mastitis. The DRB3*3 allele is associated with a lower risk of retained placenta, whereas DRB3*6 and DRB3*22 are associated with a lower risk of cystic ovarian disease. Resistance to *Dermatophilus* has also been mapped to the BoLA DR locus.

In sheep, there is an association of the class I allele SY1 with resistance to *Trichostrongylus colubriformis*. Ovar-DRB1 locus affects egg production in Ostertagia infection. Resistance to scrapie and to caseous lymphadenitis appears to be associated with possession of certain MHC class I alleles.

In goats, the class I allele Be7 is associated with resistance, and Be1 and Be14 are associated with susceptibility to caprine arthritis-encephalitis. Genetic resistance or susceptibility to *Ehrlichia ruminantium* infection (heartwater) is associated with class I CLA and Be alleles.

In horses, an allergic response to the bites of *Culicoides* midges is linked to ELA-Aw7. There is also a strong association between ELA-A3, ELA-A15, and ELA-Dw13 and the development of sarcoid tumors (fibroblastic skin tumors likely induced by bovine papilloma viruses). An autoimmune disease, equine recurrent uveitis, is strongly associated with the haplotype ELA-A9.

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In pigs, the SLA complex has an influence on major reproduction parameters such as ovulation rate, litter size, and piglet viability. This may be due to the role played by the enzyme 21-hydroxylase, whose gene is located in the class III region. Serum antibody levels are also affected in part by SLA haplotype. Even the numbers of larvae of the parasite *Trichinella spiralis* in muscle are regulated by genes in the SLA complex. Quantitative trait loci for backfat thickness, average daily gain, weight, and reproductive traits have been mapped to the SLA complex. For example, low growth performance in large white pigs is associated with possession of SLA class I alleles 4, 5, and 20. High carcass fatness in Landrace pigs is associated with alleles 1, 15, and 18. The precise gene or genes responsible for these traits has not been identified. One possible candidate is the gene that codes for a 17 β -hydroxysteroid dehydrogenase called FABGL since this enzyme oxidizes estradiol, testosterone, and dihydrotestosterone, and these hormones regulate adipose tissue formation.

Selection for specific MHC haplotypes has potential for use in developing disease-resistant strains of domestic animals. However, it must be pointed out that by selecting for a specific gene locus one may also inadvertently select for susceptibility at closely linked loci. This may outweigh the benefits of a resistant allele at one locus. An animal cannot be resistant to all infectious diseases.

9.9 MHC AND BODY ODORS

The vomeronasal organ in mammals is an olfactory organ that is used to detect information about another individual's gender, status, and individuality. The molecules that carry this information are small volatile peptides found in urine. These peptides can bind to the antigen-binding grooves of MHC class I molecules. Thus peptides known to bind to two mouse MHC class I molecules of different haplotypes were shown to induce responses (field potentials) in mouse vomeronasal organs. The responses were not haplotype specific, but different peptides induced different activation patterns. This finding may well explain how mammals such as mice can recognize the MHC of other mice by smell.

The class I region of mice, cattle, and pigs contains at least four genes coding for pheromone olfactory receptors. As a result, the MHC haplotype affects the recognition of individual odors in an allele-specific fashion and thus influences the mating preferences of mammals. Under controlled conditions, mice (and humans) prefer to mate with MHC-incompatible individuals. Such matings preferentially generate heterozygote advantage, which could lead to enhanced disease resistance. However, this type of mating could also prevent genome-wide inbreeding. Inbreeding avoidance may be the most important function of MHC-based mating preferences and therefore the fundamental selective force diversifying MHC genes in species with such mating patterns.

9.10 SOURCES OF ADDITIONAL INFORMATION

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¹⁰ CHAPTER 10 Organs of the Immune System

^{10.1} KEY POINTS

- Acquired immunity is mediated by cells called lymphocytes that are found within lymphoid organs.
- Lymphocytes arise from stem cells in the bone marrow.
- Lymphocytes mature within primary lymphoid organs. T cells mature within the thymus. B cells mature within gastrointestinal lymphoid tissues, the bone marrow, or the bursa of Fabricius, depending on the species.
- Lymphocytes bearing receptors for self-antigens are killed within primary lymphoid organs in a process called negative selection.
- Mature lymphocytes reside in secondary lymphoid organs, where their role is to encounter and respond to foreign antigens.
- The major secondary lymphoid organs include lymph nodes, spleen, bone marrow, and some Peyer's patches within the intestine.

Although antigens are trapped and processed by dendritic cells (DCs), macrophages, and B cells, acquired immune responses are actually mounted by cells called lymphocytes. Lymphocytes are the small round cells that predominate in organs such as the spleen, lymph nodes, and thymus ([Figure 10-1](#)). These are called lymphoid organs. Lymphocytes have antigen receptors on their surface, and they can therefore recognize and respond to antigens. Lymphocytes are eventually responsible for the production of antibodies and for cell-mediated immune responses. The lymphoid organs must therefore provide an environment for efficient interaction between lymphocytes, antigen-presenting cells, and foreign antigens as well as sites where lymphocytes can respond optimally to processed antigens.

Immune responses must be carefully regulated. Lymphocytes must be selected so that their receptors will only bind foreign antigens, and the response of each lymphocyte must be regulated so that it is sufficient but not excessive for the body's requirements. The lymphoid organs may therefore be classified on the basis of their roles in generating lymphocytes, in regulating the production of lymphocytes, and in providing an environment for trapping foreign antigens, processing them, and maximizing the opportunity for processed antigens to encounter and interact with antigen-sensitive cells ([Figure 10-2](#)).

^{10.2} SOURCES OF LYMPHOCYTES

Lymphoid stem cells are first produced in the fetal omentum, liver, and yolk sac. In older fetuses and in adults, these stem cells are mainly found in the bone marrow. The bone marrow has multiple functions in adult mammals. It is a hematopoietic organ containing the stem cells that give rise to all blood cells, including lymphocytes. In some mammals, such as primates, it also acts as a primary lymphoid organ (a site where newly produced lymphocytes can mature). Like the spleen, liver, and lymph nodes, the bone marrow contains many DCs and macrophages and thus removes foreign material from the blood. Finally, it contains large numbers of antibody-producing cells and is therefore a major source of antibodies. Because of these multiple functions, the bone marrow is divided into a hematopoietic compartment and a vascular compartment. These compartments alternate, like slices of cake, in wedge-shaped areas within long bones. The hematopoietic compartment contains stem cells for all the

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blood cells as well as macrophages, DCs, and lymphocytes and is enclosed by a layer of adventitial cells. In older animals these adventitial cells may become so loaded with fat that the marrow may have a fatty yellow appearance. The vascular compartment, where antigens are mainly trapped, consists of blood sinuses lined by endothelial cells and crossed by reticular cells and macrophages.

10.3 PRIMARY LYMPHOID ORGANS

The organs that regulate the development of lymphocytes are called primary lymphoid organs. Lymphocytes fall into two major populations called T cells and B cells, based on the organ in which they mature. Thus all T cells mature in the thymus. B cells, in contrast, mature within different organs, depending on the species. These include the bursa of Fabricius in birds, the bone marrow in primates and rodents, and intestinal lymphoid tissues in rabbits, ruminants, and pigs. The primary lymphoid organs all develop early in fetal life. As animals develop, newly produced, immature lymphocytes migrate from the bone marrow to the primary lymphoid organs, where they mature ([Table 10-1](#)). The primary lymphoid organs are not sites where lymphocytes encounter foreign antigens, and they do not enlarge in response to antigenic stimulation.

10.3.1 Thymus

The thymus is located in the thoracic cavity in front of and below the heart. In horses, cattle, sheep, pigs, and chickens, it also extends up the neck as far as the

FIGURE 10-1 The major lymphoid tissues of the pig, a typical mammal.

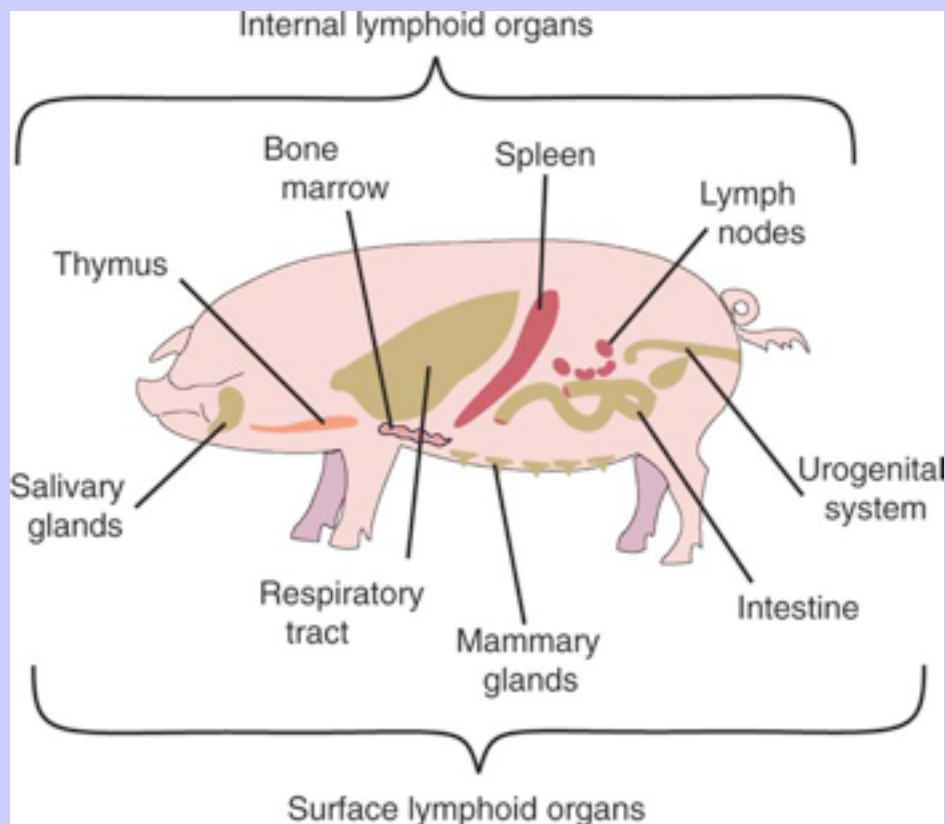
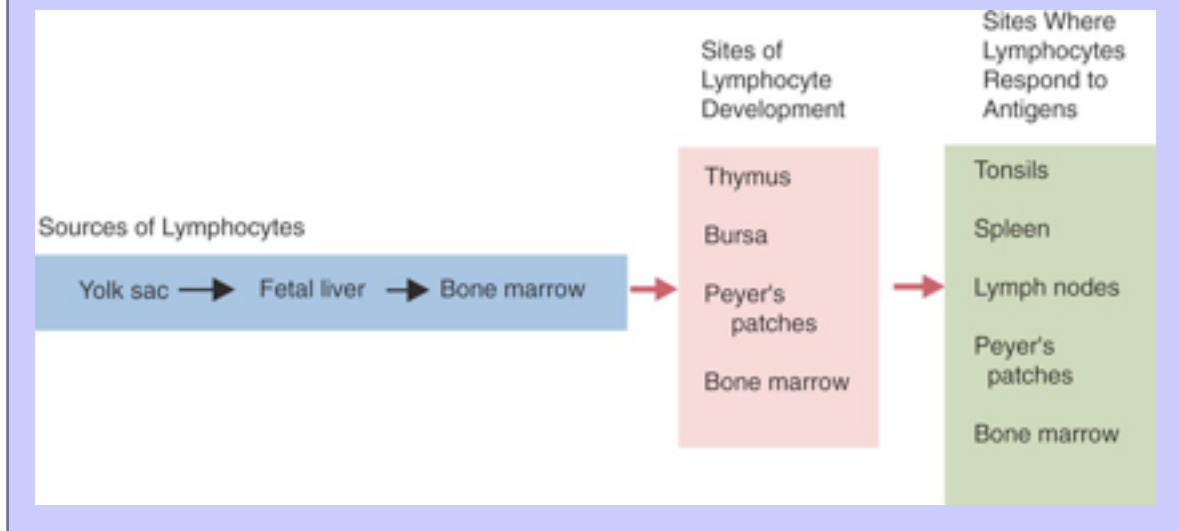
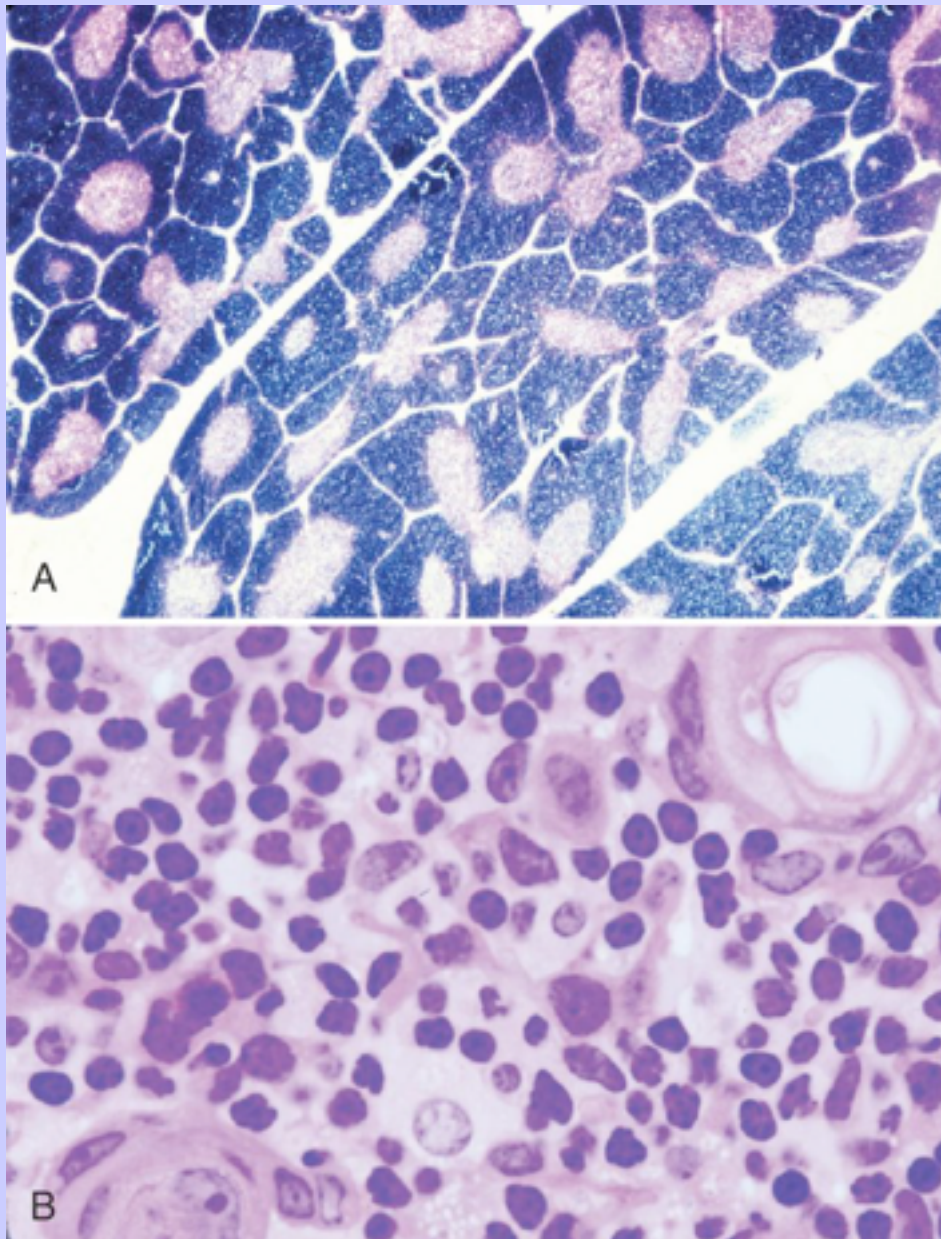


FIGURE 10-2 Role of lymphoid organs in the development and functioning of lymphocyte populations.



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FIGURE 10-3 **A**, A section of a monkey thymus. Each lobule is divided into a cortex rich in lymphocytes, hence staining darkly, and a paler medulla consisting mainly of epithelial cells. Original magnification $\times 10$. **B**, A high-power view of the medulla of a monkey thymus showing several pale-staining epithelial cells with cytoplasmic processes and many dark-staining, round lymphocytes. Original magnification $\times 1000$.



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thyroid gland. The size of the thymus varies, its relative size being greatest in the newborn animal and its absolute size being greatest at puberty. It may be very small and difficult to find in adult animals.

Table 10-1 Comparison of Primary and Secondary Lymphoid Organs

	Primary	Secondary
Origin	Ectoendodermal junction or endoderm	Mesoderm
Time of development	Early in embryonic life	Late in fetal life
Persistence	Involutes after puberty	Persists in adults
Effect of removal	Loss of lymphocytes	No or minor effects
Response to antigen	Unresponsive	Fully reactive
Examples	Thymus, bursa, some Peyer's patches	Spleen, lymph nodes

10.3.1.1 Structure

The thymus consists of lobules of loosely packed epithelial cells, each covered by a connective tissue capsule. The outer part of each lobule, the cortex, is densely infiltrated with lymphocytes (or thymocytes), but the inner medulla contains fewer lymphocytes and the epithelial cells are clearly visible ([Figure 10-3](#)). Within the medulla are also found round, layered bodies called thymic, or Hassall's, corpuscles. These contain keratin, and the remains of a small blood vessel may be found at their center. In cattle these corpuscles may contain immunoglobulin A (see [Chapter 14](#)). They stimulate thymocyte proliferation by secreting growth factors. An abnormally thick basement membrane and a continuous layer of epithelial cells surround the capillaries that supply the thymic cortex. This barrier prevents circulating foreign antigens from entering the cortex. No lymphatic vessels leave the thymus. As an animal ages, the thymus shrinks and is gradually replaced by fat. However, the aged thymus still contains small amounts of lymphoid tissue and is functionally active.

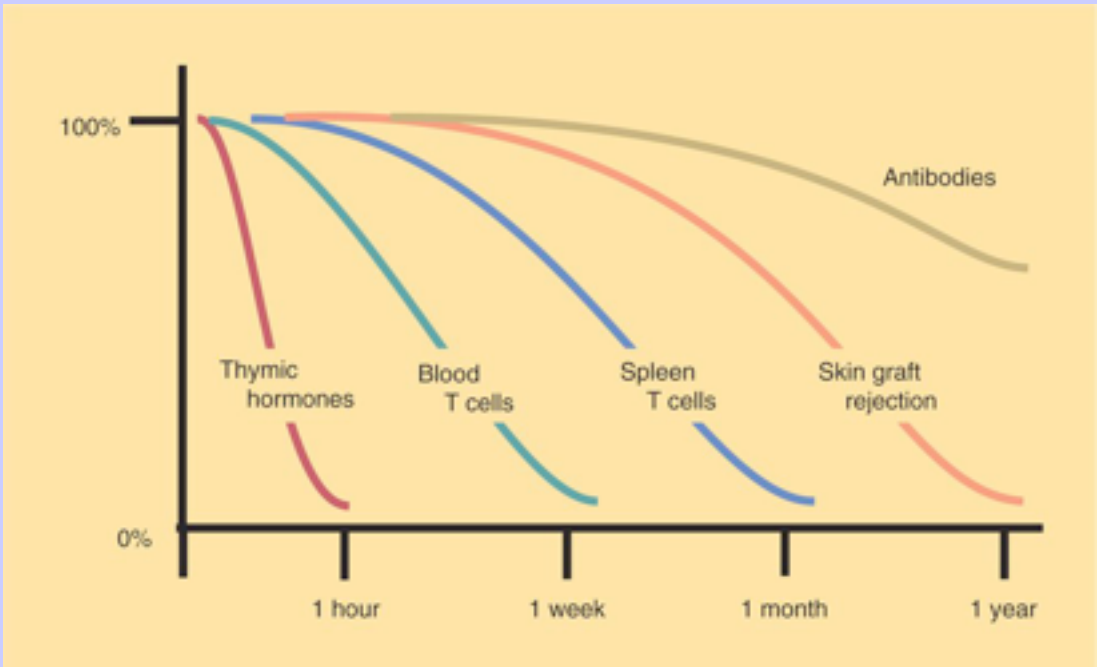
10.3.1.2 Function

The functions of the thymus are best demonstrated by studying the effects of its surgical removal in rodents. The effects of this surgery differ, depending on the age of the animal. For example, mice thymectomized within a day of birth become susceptible to infections and may fail to grow. These animals have very few circulating lymphocytes and cannot reject foreign organ grafts because they lose the ability to mount cell-mediated immune responses ([Table 10-2](#)).

Surgical removal of the thymus from adult mammals has no immediately obvious effect. But if these animals are monitored for several months, the number of lymphocytes in their blood and their ability to mount cell-mediated immune responses are gradually re-duced. This suggests that the thymus remains functional in adults, but there is a reservoir of long-lived thymus-derived cells that must be exhausted before the effects of adult thymectomy become apparent ([Figure 10-4](#)).

The results of thymectomy indicate that the neonatal thymus is the source of most blood lymphocytes

FIGURE 10-4 Effects of adult thymectomy on immune responses. Note that it takes up to a year for the full consequences to become apparent.



and that these lymphocytes are mainly responsible for mounting cell-mediated immune responses. They are called thymus-derived lymphocytes, or T cells. T cell precursors originate in the bone marrow but then enter the thymus. Once within the thymus, the cells (called thymocytes) divide rapidly. Of the new cells produced, most die by apoptosis, whereas the survivors (about 5% of the total in rodents and about 25% in calves) remain in the thymus for 4 to 5 days before leaving and colonizing the secondary lymphoid organs.

Table 10-2 Effects of Neonatal Thymectomy and Bursectomy

Function	Thymectomy	Bursectomy
Numbers of circulating lymphocytes	Disappear	No effect
Presence of lymphocytes in T dependent areas	Disappear	No effect
Graft rejection	Suppressed	No effect
Presence of lymphocytes in T independent areas	Minor depletion	Disappear
Plasma cells in lymphoid tissues	Minor drop	Disappear
Serum immunoglobulins	Minor drop	Major drop
Antibody formation	Minor effects	Major drop

T cells that enter the thymus have two conflicting tasks. They must recognize foreign antigens but at the same time must not respond strongly to normal body constituents (self-antigens). A two-stage selection process in

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the thymic medulla accomplishes this feat. Thus thymocytes with receptors that bind self-antigens strongly and that could therefore cause autoimmunity are killed by apoptosis (negative selection). Thymocytes with receptors that cannot bind any major histocompatibility complex (MHC) class II molecules and therefore cannot react to any processed antigen are also killed.

On the other hand, those thymocytes that survive the negative selection process but can still recognize specific MHC class II–antigen complexes with moderate affinity are stimulated to grow—a process called positive selection. These surviving cells eventually leave the thymus as mature T cells, circulate in the bloodstream, and colonize the secondary lymphoid organs.

Thymic epithelial cells are unusual since they express more than 400 antigens normally expressed in other tissues. This “promiscuous” gene expression ensures that developing T cells are exposed to a wide diversity of normal tissue antigens. Since T cells that respond to these antigens will die, the system ensures that those T cells leaving the thymus will not respond to these normal body components.

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10.3.1.3

Thymic Hormones

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Within the thymus, cell functions are regulated by a complex mixture of cytokines and small peptides collectively known as thymic hormones. These include peptides variously called thymosins, thymopoietins, thymic humoral factor, thymulin, and the thymostimulins. Thymulin is especially interesting because it is a zinc-containing peptide secreted by the thymic epithelial cells, and it can partially restore T cell function in thymectomized animals. Zinc is an essential mineral for the development of T cells. Consequently, zinc-deficient animals have defective cell-mediated immune responses (see [Chapter 35](#)). Hassall's corpuscles are round nests of epithelial cells scattered through the medulla of the thymus. They play a functional role in regulating thymic activity since they express a growth factor called thymic stromal lymphopoietin. This molecule activates thymic DCs so that they express high levels of the co-stimulatory molecules CD80 and CD86. As a result, these DCs can stimulate regulatory T cells. It is believed that these regulatory T cells control the positive selection of developing T cells.

10.3.2

Bursa of Fabricius

The bursa of Fabricius is found only in birds. It is a round sac located just above the cloaca ([Figure 10-5](#)). Like the thymus, the bursa reaches its greatest size in the chick about 1 to 2 weeks after hatching and then shrinks as the bird ages. It is very difficult to identify in older birds.

10.3.2.1

Structure

Like the thymus, the bursa consists of lymphocytes embedded in epithelial tissue. This epithelial tissue lines a hollow sac connected to the cloaca by a duct. Inside the sac folds of epithelium extend into the lumen, and scattered through the folds are round masses of lymphocytes called lymphoid follicles ([Figure 10-6](#)). Each follicle is divided into a cortex and a medulla. The cortex contains lymphocytes, plasma cells, and macrophages. At the corticomedullary junction there is a basement membrane and capillary network, on the inside of which are epithelial cells. These medullary epithelial cells are replaced by lymphoblasts and lymphocytes in the center of the follicle. Specialized neuroendocrine DCs of unknown function surround each follicle.

10.3.2.2

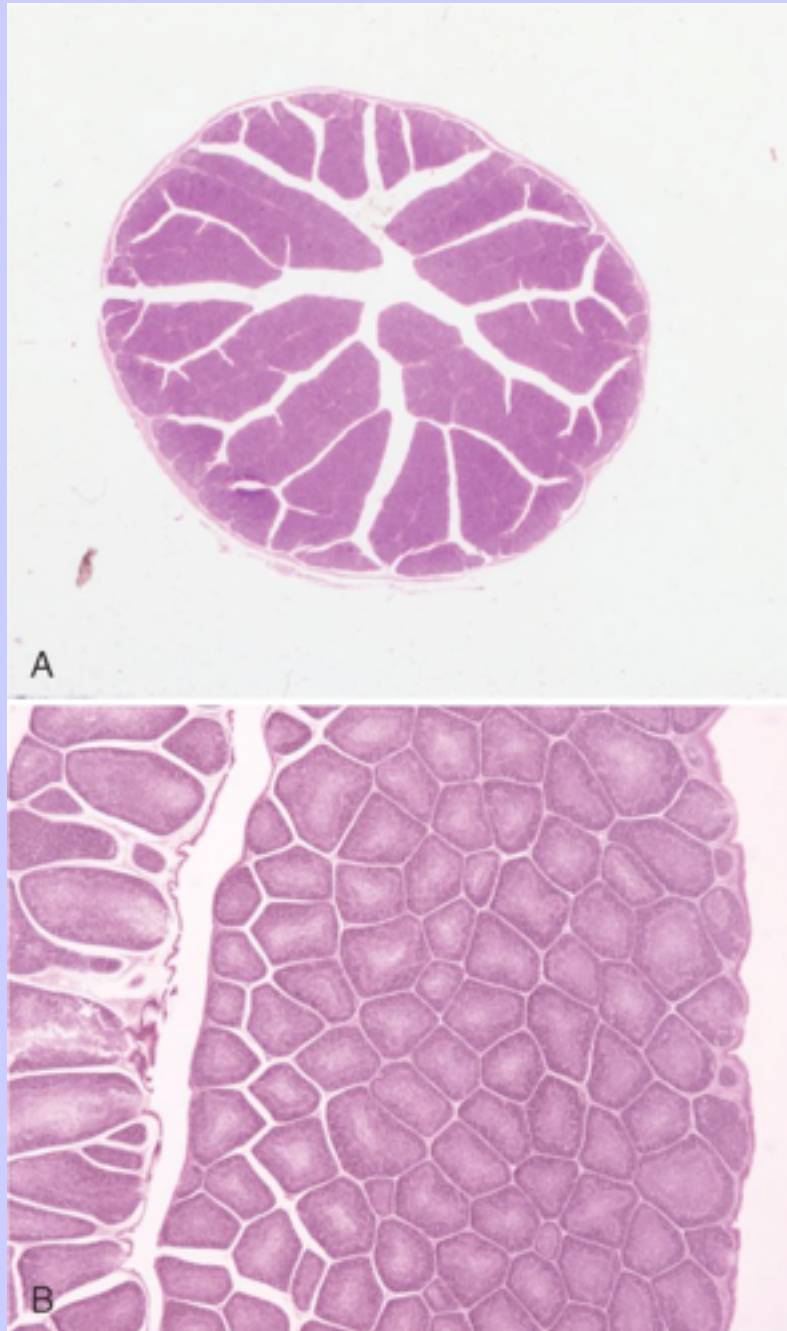
Function

The bursa may be removed either surgically or by infecting newborn chicks with a virus that destroys the bursa (infectious bursal disease virus). Since the bursa shrinks when chicks become sexually mature,

FIGURE 10-5 The bursa of Fabricius obtained from a 1-week-old chicken. It has been cut open to reveal the folds inside.



FIGURE 10-6 Photomicrographs showing the structure of the bursa of Fabricius. **A**, Low-power micrograph showing the bursa of a 13-day-old chick. Original magnification $\times 5$. **B**, A high-power view. Original magnification $\times 360$. (From a specimen provided by Drs. N.H. McArthur and L.C. Abbott.)



bursal atrophy can also be provoked by administration of testosterone. Bursectomized birds have very low levels of antibodies in their blood, and antibody-producing cells disappear from lymphoid organs. However, they still possess circulating lymphocytes and can reject foreign skin grafts. Thus bursectomy has little effect on the cell-mediated immune response. Bursectomized birds are more susceptible than normal to leptospirosis and salmonellosis but not to intracellular bacteria such as *Mycobacterium avium*.

Thus the bursa is a primary lymphoid organ that functions as a maturation and differentiation site for the cells of the antibody-forming system. Lymphocytes originating in the bursa are therefore called B cells. The bursa acts like the thymus insofar as immature cells produced in the bone marrow migrate to the bursa. These cells then proliferate rapidly, but 90% to 95% of these eventually die by apoptosis—negative selection of self-reactive B cells. Once their maturation is completed, the surviving B cells emigrate to secondary lymphoid organs.

Close examination shows that the bursa is not a pure primary lymphoid organ because it can also trap antigens and undertake some antibody synthesis. It also contains a small focus of T cells just above the bursal duct opening. Several different hormones have been extracted from the bursa. The most important of these is a tripeptide (Lys-His-glycylamide) called bursin that activates B cells but not T cells.

10.3.3 Peyer's Patches

10.3.3.1 Structure

Peyer's patches (PPs) are lymphoid organs located in the walls of the small intestine. Their structure and functions vary among species. Thus in ruminants, pigs, horses, dogs, and humans (group I), 80% to 90% of the PPs are found in the ileum, where they form a single continuous structure that extends forward from the ileocecal junction. In young ruminants and pigs the ileal PPs may be as long as 2 m. Ileal PPs consist of densely packed lymphoid follicles, each separated by a connective tissue sheath, and contain only B cells ([Figure 10-7](#)).

The ileal PPs reach maximal size and maturity before birth at a time when they are shielded from foreign antigens. The ileal PPs collectively form the largest lymphoid tissue in 6-week-old lambs. (They constitute about 1% of total body weight, like the thymus.) They disappear by 15 months of age and cannot be detected in adult sheep.

These group I species also have a second type of PP that consists of multiple discrete accumulations of follicles in the jejunum. These jejunal PPs persist for the life of the animal. They consist of pear-shaped follicles separated by extensive interfollicular tissue and contain mainly B cells with up to 30% T cells ([Figure 10-8](#)). In other mammals, such as rabbits and rodents (group II), the PPs are located at random intervals in the ileum and jejunum. In these mammals the PPs do not develop until 2 to 4 weeks after birth and persist into old age. The development of the PPs in group II animals seems to depend entirely on stimulation by the normal bacterial flora, since they remain small and poorly developed in germ-free mice. The appendix also plays a key role in B cell development in rabbits.

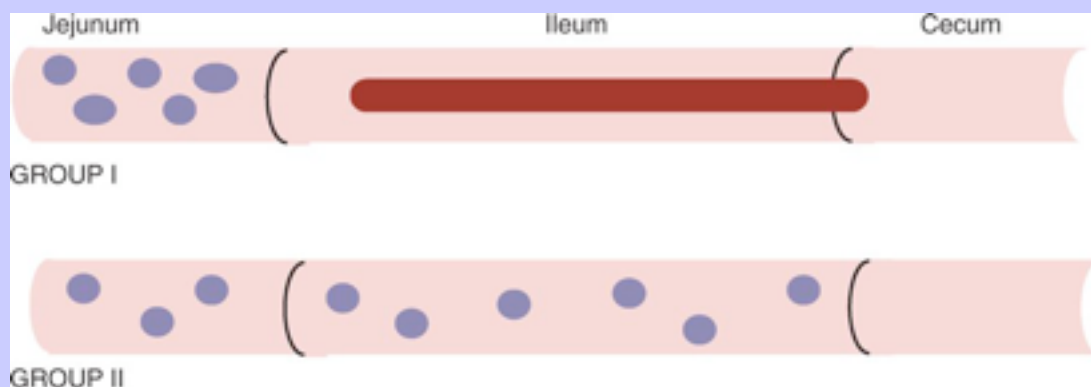
10.3.3.2 Function

The behavior of the B cells in the ileal PPs of group I species resembles that of B cells in the avian bursa. Thus ileal PPs are sites of rapid B cell proliferation, although most cells then undergo apoptosis and the survivors are released into the circulation. If their ileal PPs are surgically removed, lambs become B cell deficient for at

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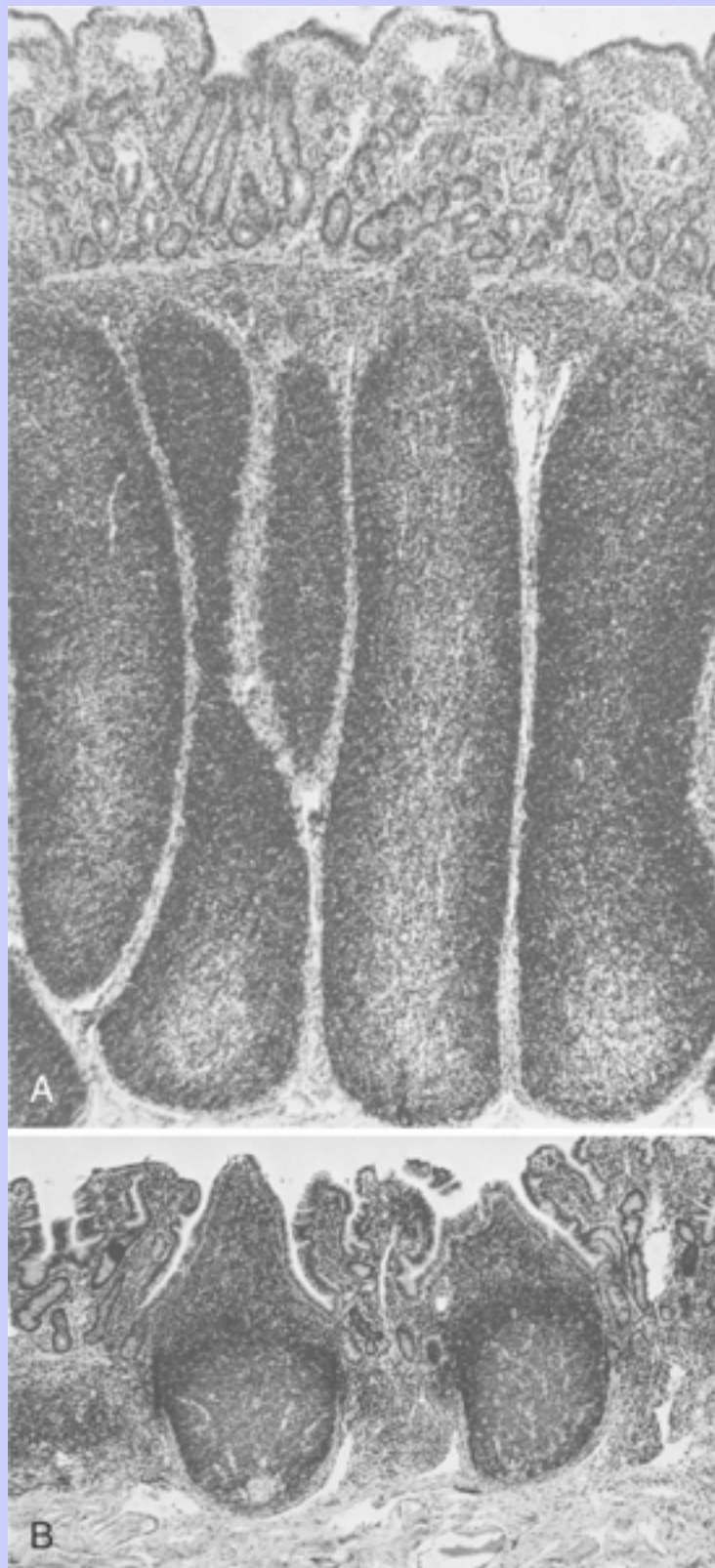
least a year and fail to produce antibodies. The bone marrow of lambs contains many fewer lymphocytes than the bone marrow of laboratory rodents, and their ileal PPs are therefore their most significant source of B cells. The group I ileal PPs are therefore

FIGURE 10-7 Schematic diagram showing the differences between the arrangement of Peyer's patches (PPs) in group 1 and group II mammals. The large ileal PP in group I mammals is a primary lymphoid organ that regresses at about a year of age.



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FIGURE 10-8 Structure of the two different types of Peyer's patch (PP) in sheep. **A**, An ileal PP at age 8 weeks. **B**, A PP from the jejunum, also at 8 weeks. Original magnification $\times 32$. (From Reynolds JD, Morris B: *Eur J Immunol* 13:631, 1983.)



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primary lymphoid organs that serve a function similar to that of the bursa of Fabricius.

The pig is also a group I species. Pigs have about 30 jejunal PPs that are of conventional structure and a single, large ileal PP. The ileal PP lacks T cells and has a structure similar to that seen in sheep. It regresses within the first year of life, so it is likely that it is a primary lymphoid organ.

Dogs also belong to group I. They have two types of PPs, including a single ileal PP that shows early involution and contains predominantly immature B cells.

10.3.4 Lymphoglandular Complexes

Lymphoglandular complexes are found in the wall of the large intestine and cecum in horses, ruminants, dogs, and pigs. They consist of submucosal masses of lymphoid tissue penetrated by radially branching extensions of mucosal glands. These glands penetrate both the submucosa and the lymphoid nodule. They are lined by intestinal columnar epithelium containing goblet cells, intraepithelial lymphocytes, and M cells (see [Chapter 19](#)). Their function is unknown, but they contain many plasma cells, suggesting that they are sites of antibody production. Because of their structural similarity to the avian bursa and the presence of many M cells, they may be antigen-sampling sites.

10.3.5 Bone Marrow

The specialized ileal PP is the primary lymphoid organ for B cells only in group I mammals (ruminants, pigs, and dogs). In group II mammals the bone marrow probably serves this function. There is no exclusive B cell development site in the bone marrow, although it is suggested that precursor B cells develop at the outer edge of the marrow and migrate to the center as they mature and multiply. Negative selection apparently occurs within the bone marrow so that most of the pre-B cells generated are destroyed.

10.4 SECONDARY LYMPHOID ORGANS

In contrast to the primary lymphoid organs, the secondary lymphoid organs arise late in fetal life and persist in adults. Unlike primary lymphoid organs, they enlarge in response to antigenic stimulation. Surgical removal of a secondary lymphoid organ does not significantly reduce immune capability. Examples of secondary lymphoid organs include the spleen, the lymph nodes, the tonsils, and other lymphoid tissues in the intestinal, respiratory, and urogenital tracts. These organs contain DCs that trap and process antigens and lymphocytes that mediate the immune responses. The overall anatomical structure of these organs is therefore designed to facilitate antigen trapping and to provide maximal opportunities for processed antigens to be presented to lymphocytes.

10.4.1 Lymph Nodes

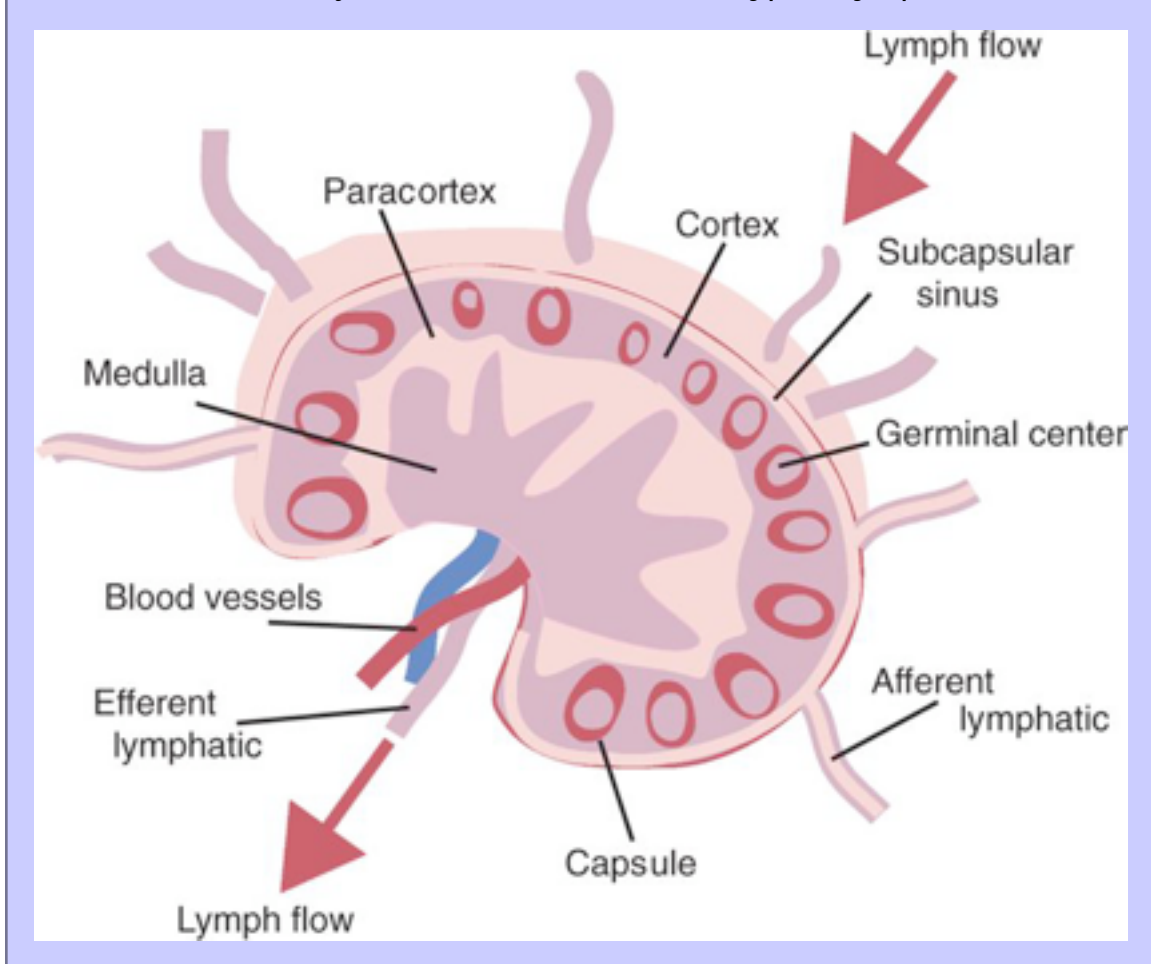
10.4.1.1 Structure

Lymph nodes are round or bean-shaped filters strategically placed on lymphatic vessels in such a way as to trap antigens carried in lymph ([Figure 10-9](#)). Lymph nodes consist of a reticular network filled with lymphocytes, macrophages, and DCs through which lymphatic sinuses penetrate ([Figure 10-10](#)). A subcapsular sinus is located immediately under the connective

FIGURE 10-9 Lateral view of the head of a bovine showing the way in which the lymphatics drain to the parotid lymph node. (From Sisson S [revised by Grossman JD]: *Anatomy of the domestic animals*, ed 4, Philadelphia, 1953, Saunders.)



FIGURE 10-10 The major structural features of a typical lymph node.



tissue capsule of the node. Other sinuses pass through the body of the node but are most prominent in the medulla. Afferent lymphatics enter the node around its circumference, and efferent lymphatics leave from a depression or hilus on one side. The blood vessels supplying a lymph node also enter and leave through the hilus.

The interior of a lymph node is divided into a peripheral cortex, a central medulla, and an ill-defined region between these two regions called the paracortex ([Figure 10-11](#)). B cells predominate in the cortex, where they are arranged in nodules. In lymph nodes that have been stimulated by antigen, some of the cells within these nodules expand to form foci of dividing cells called germinal centers ([Figure 10-12](#)). Germinal centers have light and dark zones. The dark zones are sites where B cells proliferate and undergo a process called somatic mutation (see [Chapter 15](#)). The light zones are sites where immunoglobulin class switching and memory B cell formation occur (see [Chapter 13](#)). A few T cells are found in the cortex, in the region immediately surrounding each germinal center.

T cells and DCs predominate in the paracortex. In neonatally thymectomized or congenitally athymic animals this zone loses cells and is said to be a T-dependent region ([Figure 10-13](#)). Many different cell types are found in the medulla. They include reticulum cells that generate the fibrillar scaffold, DCs and macrophages that trap

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antigens, and B cells and plasma cells that produce antibodies. These cells are arranged in cellular cords between the lymphatic sinuses.

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FIGURE 10-11 A section of bovine lymph node. Original magnification $\times 12$.
(From a specimen provided by Dr. W.E. Haensly.)

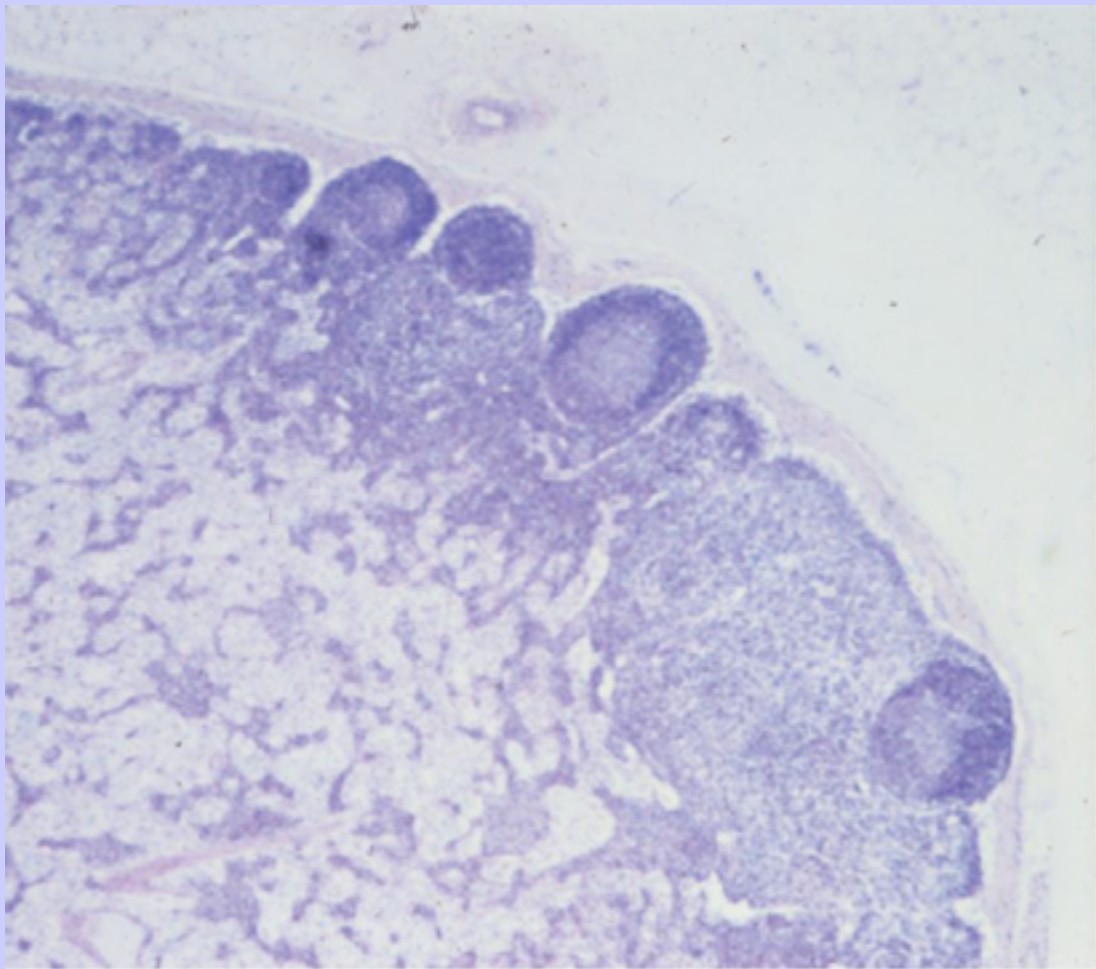


FIGURE 10-12 A germinal center in the cortex of a cat's lymph node. Note the large, pale area in the center of the germinal center where the cells are dividing. Original magnification $\times 120$. (From a specimen provided by Dr. W.E. Haensly.)

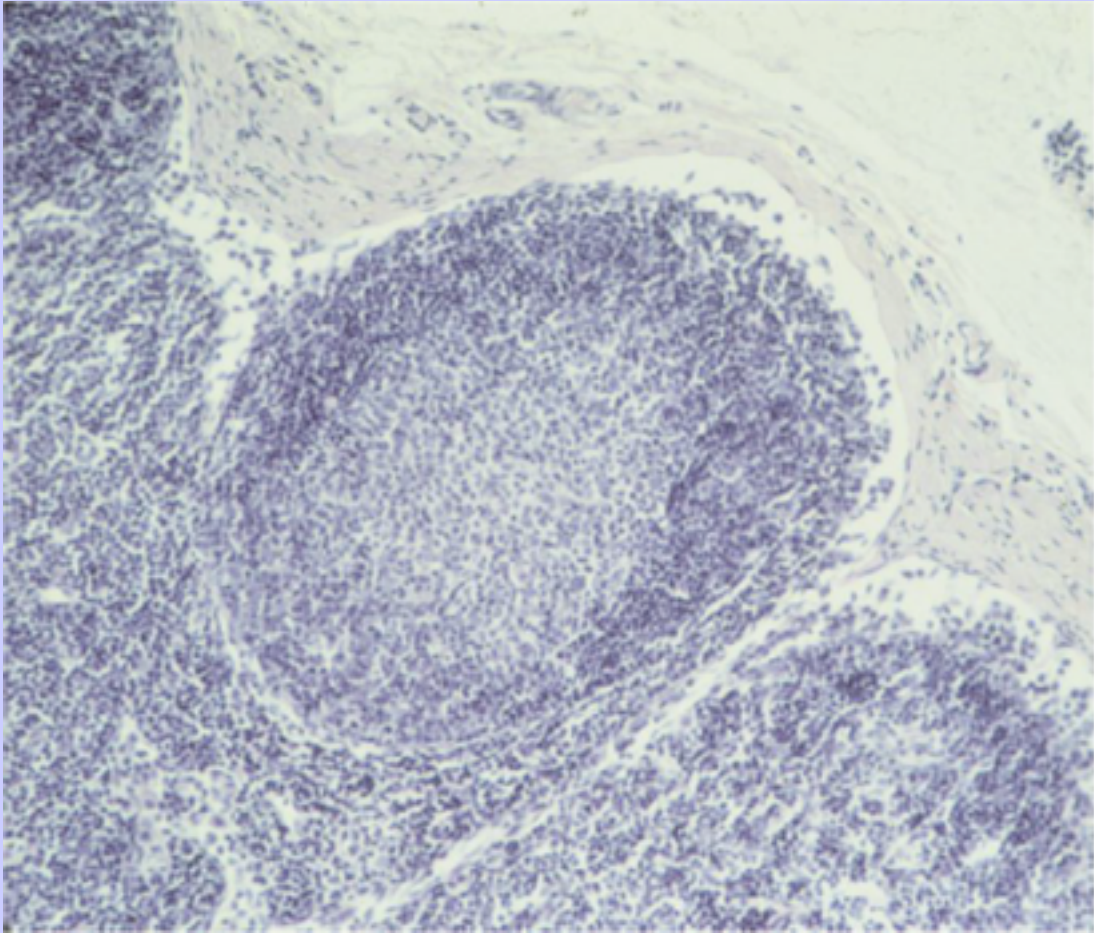
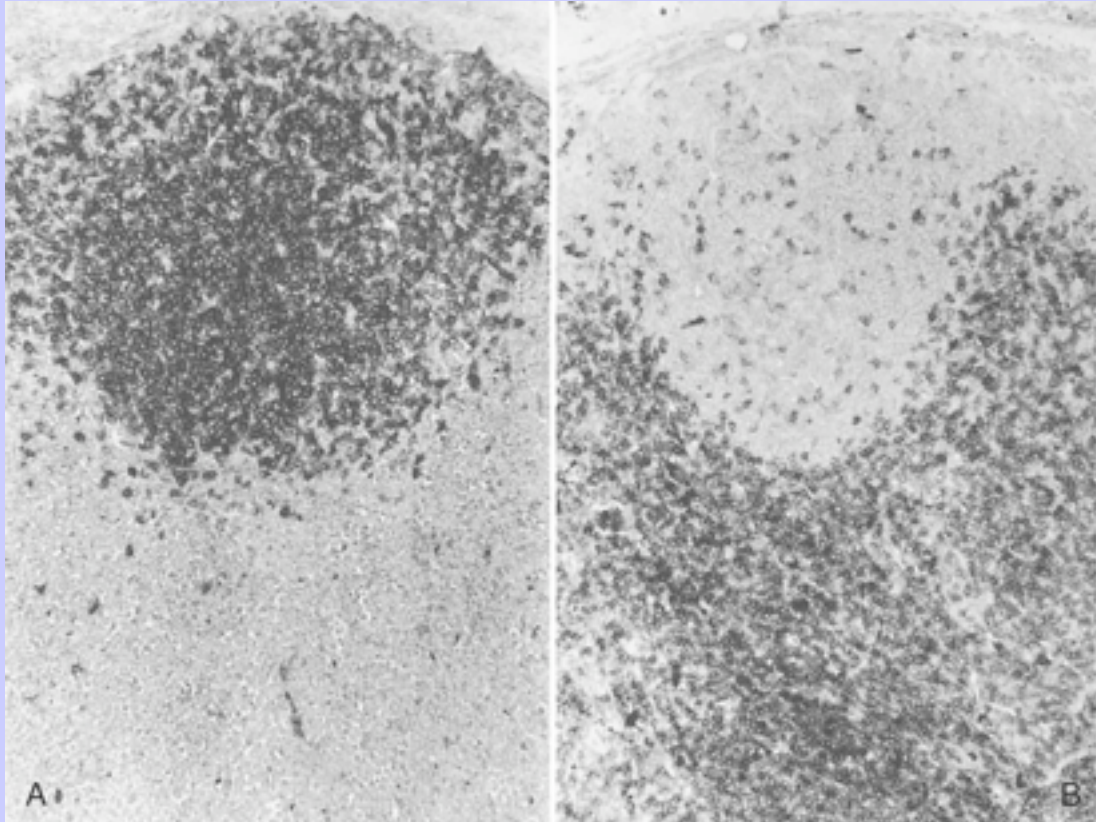


FIGURE 10-13 Normal bovine lymph node stained by the immunoperoxidase technique (see [Chapter 38](#)) with **(A)** a monoclonal antibody that identifies B lymphocytes and **(B)** a monoclonal antibody that identifies T lymphocytes. (Courtesy Drs. I. Morrison and N. MacHugh.)



Lymph nodes are very busy places, with cells coming and going in response to a multitude of signals. One obvious question therefore is how these signals are delivered. It now appears that the reticular fibers that were known to provide the structural scaffolding of the lymph node also serve as conduits for the transmission of signals. Soluble molecules are transmitted through these very small tubes and are encountered by conduit-associated DCs. Gaps in the conduit are “covered” by DCs, which can then sense the contents of conduit fluid. The conduits are linked to afferent lymphatics so that soluble antigens can reach deep into a node long before antigen-laden DCs migrate in.

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The principal function of a secondary lymphoid organ such as a lymph node is to facilitate the interactions between antigen-presenting cells and antigen-sensitive T and B cells. These cells often travel large distances and are then guided to their appropriate contacts with great precision. A complex mixture of small proteins, the chemokines, guides these cells. Thus chemokines, together with adhesion molecules, drive the emigration of lymphocytes from high endothelial venules (HEVs) into the lymph node. Once they enter the lymph node, T cells and B cells are guided to their respective regions by chemokines secreted by lymph node stromal cells

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and from follicular and interdigitating DCs. Immature DCs, once they encounter antigen, are guided into lymph nodes by chemokines. Thus DCs are attracted to the paracortex, where they present their antigen to T cells. Once this is accomplished, the DCs change their chemokine receptor phenotype and so leave the node. Soluble antigen entering the node may be taken up by DCs, macrophages, or B cells. The activated T cells can interact with the B cells and trigger antibody formation and the production of plasma cells and memory cells. Activated T cells and B cells migrate towards the lymph node marginal zone, where they interact.

Chemokines control the relocation and recirculation of lymphocytes and ensure that the correct lymphocytes end up in the correct positions. For example, T cells expressing CCR7 are attracted to the perifollicular area of the cortex, where the chemokines CCL19 and CCL21 are produced. B cells, on the other hand, express CXCR5 and are attracted to the interior of germinal centers, where its ligand, the chemokine CXCL13, is produced. When T cells are activated, they too may express CXCR5 and so enter germinal centers where they “help” B cells respond to antigens.

Other lymphoid organs employ other homing receptors. Thus the homing receptor MAdCAM-1 is expressed in blood vessels in intestinal lymphoid tissues such as PPs. Lymphocytes that recirculate to the intestine tend to express high levels of the integrin $\alpha_4\beta_7$, the ligand for MAdCAM-1.

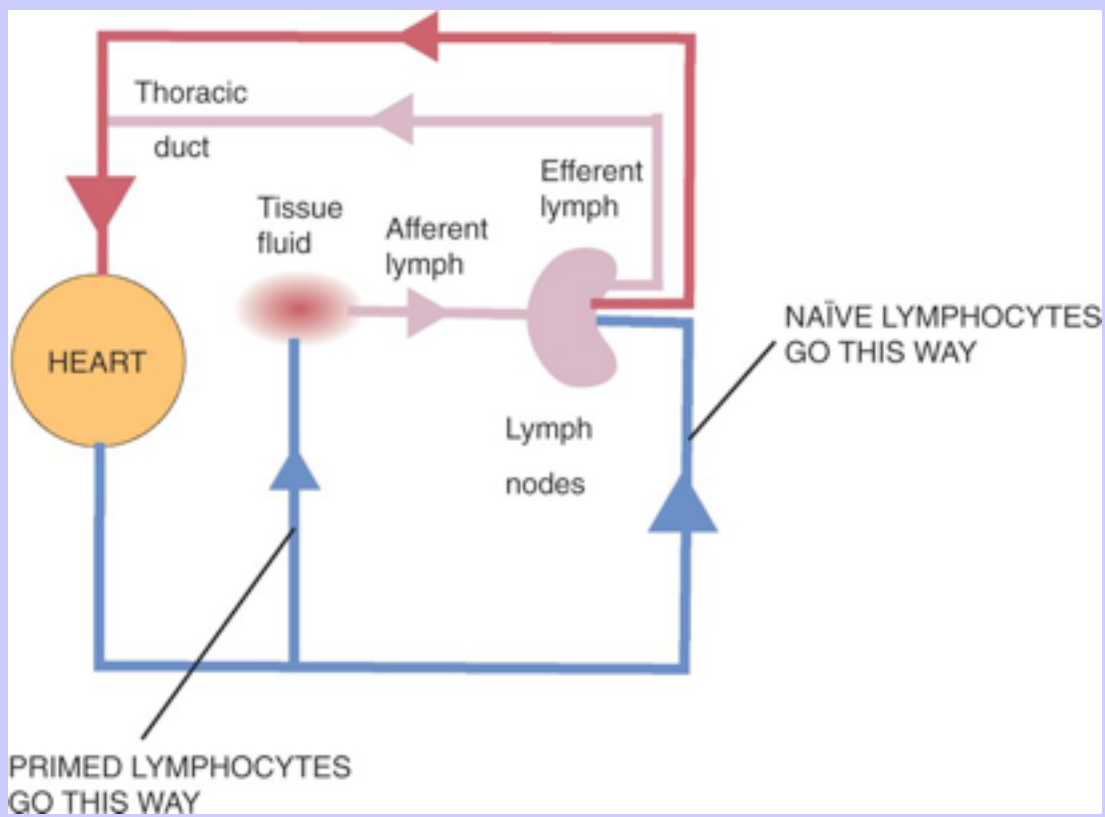
10.4.1.2

Lymphocyte Circulation

T cells constantly circulate around the body in the blood and tissue fluid and are the predominant lymphocytes in blood ([Figure 10-14](#)). As they travel, they survey the body for foreign antigens and preferentially home in to sites of microbial invasion and inflammation. They also spend time in the secondary lymphoid organs.

Circulating T cells leave the bloodstream by two routes. T cells that have not encountered antigens previously (“naïve” T cells) bind to venules in the paracortex of lymph nodes. These are called high endothelial venules (HEVs) because they are lined with tall, rounded endothelial cells ([Figure 10-15](#)) quite unlike the flattened endothelium found in other blood vessels. The high endothelial cells are not joined by tight junctions but are linked by discontinuous “spot-welded” junctions. This means that lymphocytes can

FIGURE 10-14 Circulation of lymphocytes. T cells circulate in both the bloodstream and the lymphatic fluid. Their precise route through a lymph node depends on whether they are naïve or primed. Thus naïve lymphocytes enter lymph nodes through the bloodstream and the high endothelial venules. Primed lymphocytes, in contrast, migrate through the tissues and so enter through afferent lymphatics. They all leave through efferent lymphatics.



pass easily between the high endothelial cells. Circulating lymphocytes can adhere to these high endothelial cells and then migrate into the paracortex. The emigration of lymphocytes from HEVs resembles that of neutrophils in inflamed blood vessels. Thus the cells first roll along the endothelial surface binding to selectins. For example, L-selectin (CD62L) on lymphocytes binds to receptors such as GlyCAM-1 or CD34 (sialomucin) on the cells lining the HEVs ([Figure 10-16](#)). As they roll, the lymphocytes become activated and express integrins. This results in their complete arrest and emigration. The number and length of HEVs are variable and controlled by local activity. Thus stimulation of a lymph node by the presence of antigens results in a rapid increase in the length of its HEVs. If, however, a lymph node is protected from antigens, its HEVs shorten. HEVs are not normally found in sheep lymph nodes.

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In contrast to naïve T cells, memory T cells leave the bloodstream through conventional blood vessels in tissues and are then carried to lymph nodes by tissue fluid (afferent lymph). Up to 90% of lymphocytes leaving a node are derived from cells entering through HEVs whereas about 10% enter through afferent lymph.

Lymph flows from the tissues to lymph nodes through afferent lymphatics. It leaves the lymph nodes by efferent lymphatics. Typically, afferent lymph in sheep contains 85% T cells, 5% B cells, and 10% DCs. Efferent lymph contains greater than 98% lymphocytes of which 75% are T cells and 25% are B cells. The efferent lymphatics eventually join together to form large lymph vessels. The largest of these lymph vessels is the thoracic duct, which drains the lymph from the lower body and intestine and empties it into the anterior vena cava. If the thoracic duct is cannulated and the lymph removed, blood lymphocyte numbers (essentially all T cells) drop significantly within a few hours. The T cells also disappear from the paracortex of lymph nodes. This rapid depletion of T cells implies that thoracic duct lymphocytes normally circulate back to lymph nodes through the blood.

10.4.1.3

Species Differences

Domestic pigs and related swine, hippopotamuses, rhinoceroses, and some dolphins are different. Their lymph nodes consist of several lymphoid “nodules” oriented so that the cortex of each nodule is located toward the center of the node whereas the medulla is at the periphery ([Figure 10-17](#)). Each nodule is served by a single afferent lymphatic that enters the central cortex as a lymph sinus. Thus afferent lymph is carried deep into the node. A cortex surrounds the lymph sinus. Outside this region are a paracortex and a medulla. This medulla may be shared by adjacent nodules ([Figure 10-18](#)). Lymph passes from the cortex at the center of the node to the medulla at the periphery before leaving through the efferent vessels that drain the region between nodules. The cortex and paracortex have a similar structure to that seen in other mammals. The medulla has very few sinuses but consists of a dense mass of cells that is relatively

FIGURE 10-15 A section of human tonsil showing a high endothelial venule with its characteristic high, rounded endothelial cells. Note the lymphocytes emigrating between the endothelial cells.

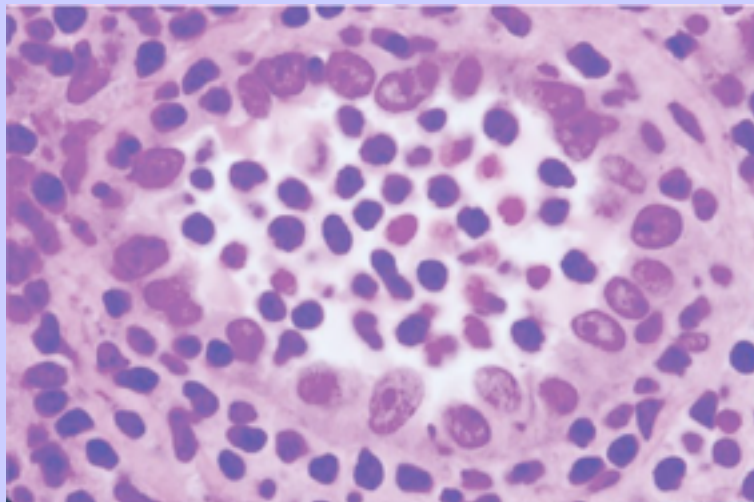
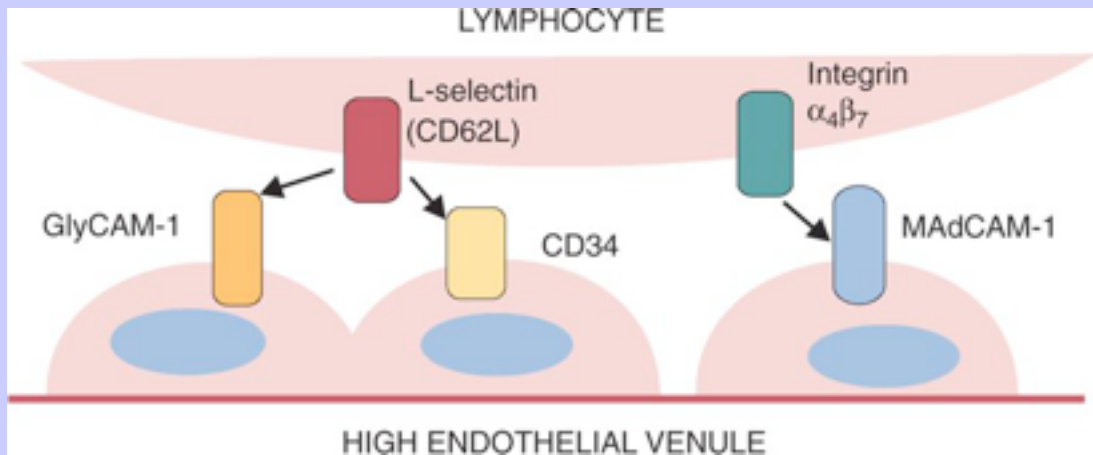


FIGURE 10-16 Binding of circulating lymphocytes to ligands on endothelial cells in high endothelial venules is brought about mainly by L-selectin binding to GlyCAM-1 and CD34. In the intestine, lymphocytes bearing the integrin $\alpha_4\beta_7$ bind to MAdCAM-1 endothelial cells.



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FIGURE 10-17 Structure of a pig lymph node. Compare this with [Figure 10-18](#).

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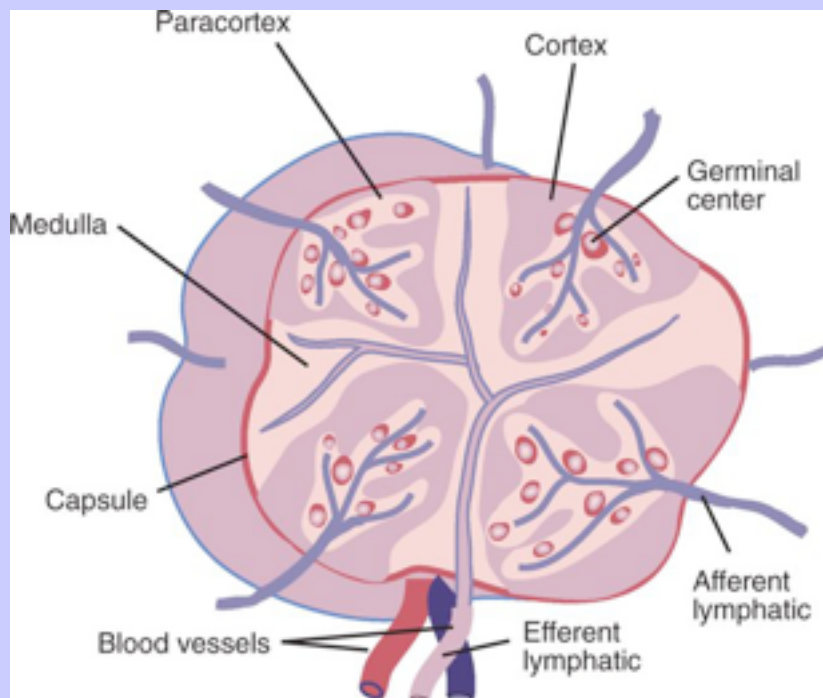


FIGURE 10-18 A section of a pig lymph node. Note how the germinal centers are located in the interior of the node. Original magnification $\times 12$. (From a specimen provided by Drs. N.H. McArthur and L.C. Abbott.)

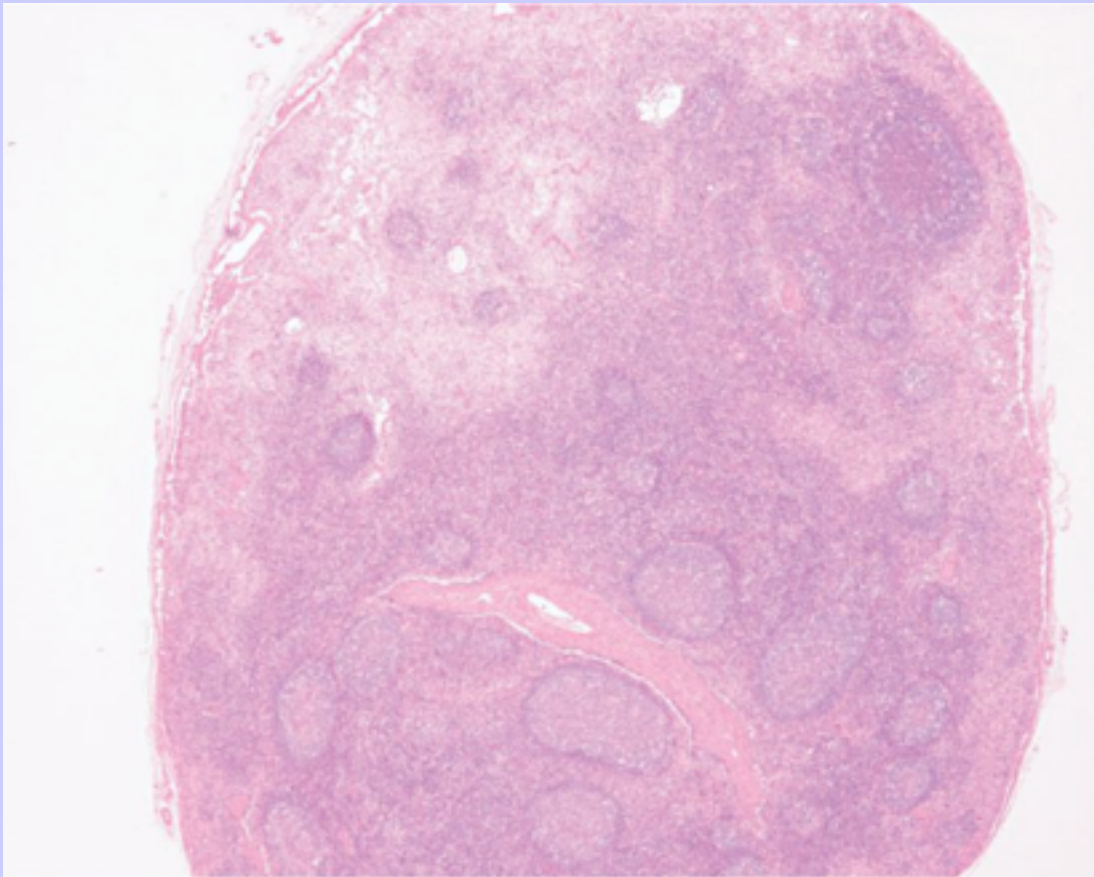
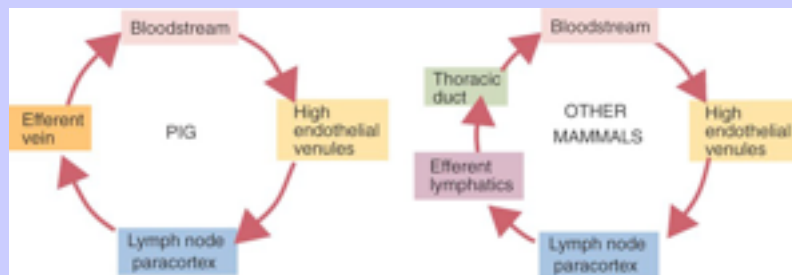


FIGURE 10-19 Comparison between the major route of circulation of T cells in the pig and in other mammals. Note that pig lymphocytes are largely confined to the bloodstream.



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impermeable to cells in the lymph. As a result, few cells migrate through the medulla. T cells in these species enter the lymph node in the conventional way through HEVs. However, they do not leave the lymph node through the lymphatics but migrate directly back to the bloodstream through the HEVs of the paracortex ([Figure 10-19](#)). Very few lymphocytes are found in pig lymph.

In marine mammals, lymph node structure is highly variable. Thus all the lymph nodes of bottlenose dolphins are of conventional structure. In striped dolphins, on the other hand, some lymph nodes (mesenteric, for example) are of conventional structure while others (mediastinal) have the inverted structure described above. Thus both forms of lymph node can be present in a single individual.

10.4.1.4

Response to Antigens

When microbes invade tissues, the resident DCs are activated and migrate to the draining lymph node, where they accumulate in the paracortex and cortex. These DCs form a web through which antigens must pass. Antigens are captured and then presented by these DCs to T cells. The T cells are initially activated in the paracortex, whereas the B cells remain randomly dispersed in the primary follicles. Both cell populations then migrate to the edges of the follicles, where they interact. Once antibody production is stimulated, the progeny of these B cells move to the medulla and begin to secrete antibodies. Some of these antibody-producing cells may escape into the efferent lymph and colonize downstream lymph nodes. Several days after antibody production is first observed in the medulla, germinal centers appear in the cortex.

Adherence to follicular DCs is the predominant means of antigen trapping once an animal has been sensitized by previous exposure to an antigen. In a secondary response the germinal centers become less obvious as activated memory cells emigrate in the efferent lymph. Once this stage is completed, the germinal centers redevelop.

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Antigen-stimulated lymph nodes trap lymphocytes. Local interactions between infectious agents and mast cells result in the production of tumor necrosis factor- α (TNF- α). The TNF- α blocks the passage of lymphocytes through these organs, the lymphocytes accumulate, and the lymph nodes swell. This trapping concentrates lymphocytes close to sites of antigen accumulation. After about 24 hours the lymph nodes release their trapped cells. As a result, their cellular output is increased for several days.

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When responding to antigens that stimulate a cell-mediated rather than an antibody-mediated immune response, such as a skin graft, the T cell-rich paracortical areas produce large pyroninophilic cells. Pyronin is a stain for RNA; thus a cell with pyroninophilic cytoplasm is rich in ribosomes and is probably a protein-producing cell. These large pyroninophilic cells give rise, in turn, to the T cells that participate in the cell-mediated immune responses.

10.4.2

Hemolymph Nodes

Hemolymph nodes are structurally similar to lymph nodes found in association with the blood vessels of ruminants and other mammals. Their function is unclear. They differ from conventional lymph nodes in that their lymphatic sinuses contain numerous red cells. They have a cortex containing germinal centers and B cells. T cells predominate at the center in association with lymphatic sinuses. There are some differences, however, in the characteristics of these T cells as compared with conventional lymph nodes (more γ/δ^+ cells and WC1 $^+$ T cells; fewer CD8 $^+$ T cells) (see [Chapter 12](#)). Intravenously injected carbon particles are trapped in the sinusoids of hemolymph nodes, suggesting that they may combine features of both the spleen and lymph nodes.

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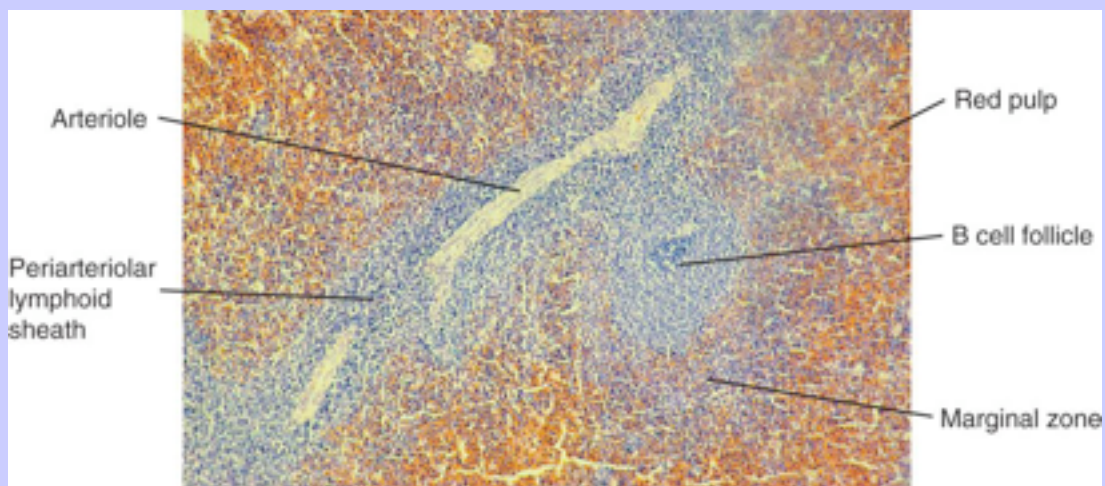
10.4.3 Spleen

Just as lymph nodes filter antigens from lymph, so the spleen filters blood. Indeed, the spleen can be considered a specialized lymph node for blood-borne antigens. The filtering process removes both antigenic particles such as blood-borne microorganisms, cellular debris, and aged blood cells. This filtering function together with highly organized lymphoid tissue makes the spleen an important component of the immune system. In addition to its immune functions, the spleen also stores red cells and platelets, recycles iron, and undertakes red cell production in the fetus. As a result, the spleen consists of two forms of tissue. One, called the red pulp, is used predominantly for blood filtering and for red cell storage. The other is rich in lymphocytes, where immune responses occur, and is called the white pulp.

10.4.3.1 Structure of White Pulp

Vessels entering the spleen travel through muscular trabeculae before entering its functional areas. Immediately on leaving the trabeculae, these arterioles branch and each is surrounded by a layer or sheath of lymphoid tissue known, naturally enough, as the periarteriolar lymphoid sheath (Figure 10-20). The arteriole eventually leaves this sheath and branches into penicillary arterioles. In some, but not all, mammals, these penicillary arterioles are surrounded by ellipsoids (periarteriolar macrophage sheaths). These arterioles then open, either directly or indirectly, into venous sinuses that drain into the splenic venules. Ellipsoids are relatively large and prominent in pigs, mink, dogs, and cats, are small and indistinct in horses and cattle, but are absent in laboratory animals such as the mouse, rat, guinea pig, and rabbit. In species that lack ellipsoids, particles appear to be

FIGURE 10-20 Histological section showing the structure of the bovine spleen. Original magnification $\times 50$. (From a specimen provided by Dr. J.R. Duncan.)



trapped primarily in the marginal zone of the white pulp.

The white pulp contains both B and T cells which accumulate in their specific zones under the influence of many different chemokines. The periarteriolar lymphoid sheaths consist largely of T cells. The B cell areas, in

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contrast, consist of round primary follicles scattered through the sheaths. Within the T cell areas, the T cells interact with DCs and passing B cells. The B cell follicles are regions where clonal expansion, isotype switching, and somatic hypermutation occur. Following antigenic stimulation, these follicles develop germinal centers.

The white pulp is separated from the red pulp by a marginal sinus, a reticulum sheath, and a marginal zone of cells. This marginal zone is an important transit area for white cells moving between the blood and the white pulp. In addition, it is rich in macrophages, DCs, and B cells. Most of the blood that enters the spleen flows into the marginal sinus and through the marginal zone before returning to the circulation through venous sinuses. This flow pattern ensures that these antigen-presenting cells can capture any circulating antigens and deliver them to the lymphoid cells of the white pulp. The white pulp is involved in acquired immune responses, while the cells of the marginal zone can participate in both innate and acquired immune responses. White pulp does not contain HEVs. Instead, lymphocytes enter the white pulp through the marginal zone, although the route by which they leave is unclear.

10.4.3.2

Response to Antigen

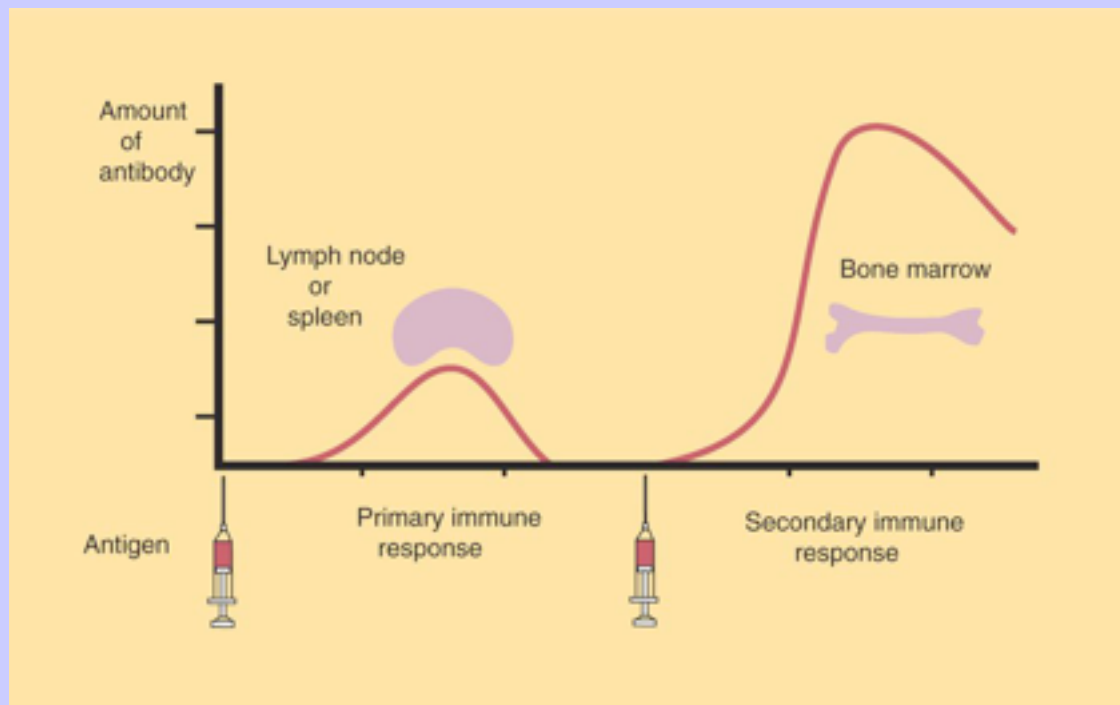
Intravenously administered antigens are trapped in the spleen. Depending on the species, they are either taken up by DCs in the marginal zone or in the peri-arterial macrophage sheaths. These DCs and macrophages carry the antigen to the primary follicles of the white pulp from which, after a few days, antibody-producing cells migrate. These antibody-producing cells (plasma cells and plasmablasts) colonize the marginal zone and move out into the red pulp. Antibodies produced by these cells move rapidly into the bloodstream. Germinal center formation also occurs in the primary follicles. In an animal possessing circulating antibody, trapping by DCs within the follicles becomes significant. As in a primary immune response, the antibody-producing cells migrate from these follicles into the red pulp and the marginal zone, where antibody production occurs, although some antibodies may also be produced within the follicles.

10.4.4

Other Secondary Lymphoid Organs

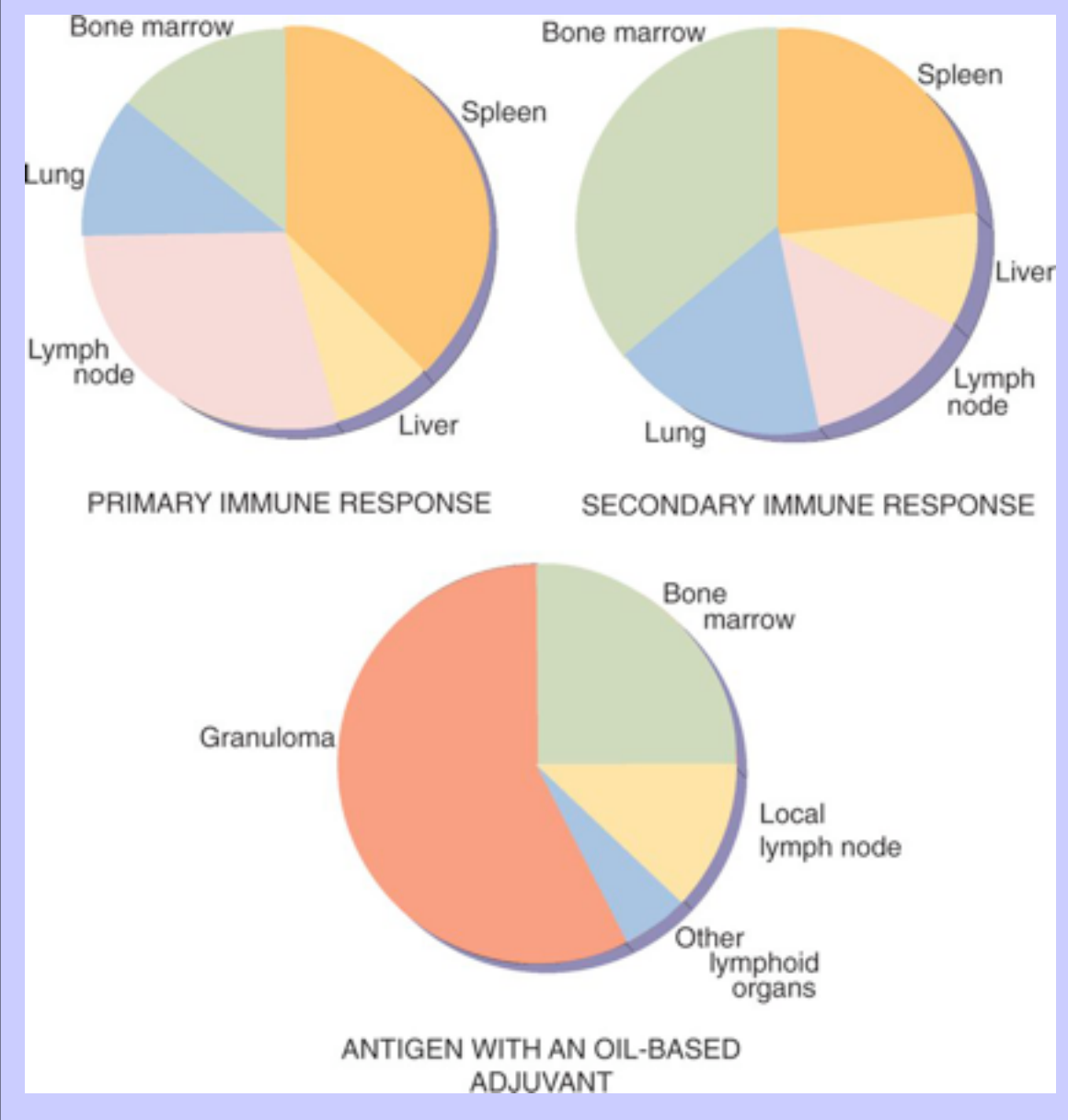
Sites where antibodies are produced include not only the spleen and lymph nodes but also the bone marrow, tonsils, and lymphoid tissues scattered throughout the body, most notably in the digestive, respiratory, and urogenital tracts. Although its scattered nature makes it difficult to measure, the bone marrow is the largest mass of secondary lymphoid tissue in an adult. If antigen is given intravenously, much will be trapped not only in the liver and spleen but also in the bone marrow. However, during a primary immune response, antibodies are largely produced in the spleen and lymph nodes ([Figure 10-21](#)). Toward the end of that response, memory cells leave the spleen and colonize the bone marrow. When a second dose of an antigen is given, the bone marrow produces very large quanti

FIGURE 10-21 Although the primary immune response to intravenously injected antigen takes place in the lymph nodes or spleen, the antibodies produced in a secondary response are largely produced in the bone marrow.



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FIGURE 10-22 Relative contribution of different organs or tissues to antibody production after administration of antigen either intravenously or intramuscularly with Freund's complete adjuvant. The adjuvant (see [Chapter 20](#)) causes the accumulation of lymphocytes and antigen-processing cells. It thus forms a lymphoid nodule where antibodies are produced.



ties of antibody and is the major source of antibodies in adult rodents. Up to 70% of the antibody to some antigens may be produced by cells in the bone marrow ([Figure 10-22](#)).

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11 CHAPTER 11 Lymphocytes

11.1 KEY POINTS

- Lymphocytes are the cells that can recognize and respond to foreign antigens.
- Lymphocytes all look the same but can be differentiated by their characteristic cell surface molecules.
- These cell surface molecules are classified by the cluster of differentiation system.
- Lymphocytes possess surface molecules that are involved in signaling from the antigen receptors.
- They possess receptors for cytokines, immunoglobulins, and complement.
- In domestic animal species, some cell surface molecules are unique to each species. These are classified by the workshop cluster system.
- The collection of cell surface molecules on a lymphocyte is called its immunophenotype. They can be detected and measured using an instrument called a flow cytometer.

Lymphocytes play key roles in the defense of the body. There are three major types of lymphocyte. These are natural killer (NK) cells that play a role in innate immunity; T cells that regulate acquired immunity and are responsible for cell-mediated immunity; and B cells that are responsible for antibody production. Within these major types are many cell subpopulations, each with different characteristics and functions. This chapter reviews the structure and properties of these lymphocytes and some important subpopulations.

11.2 LYMPHOCYTE STRUCTURE

Lymphocytes are small, round cells, 7 to 15 μm in diameter. Each contains a large, round nucleus that stains intensely and evenly with hematoxylin ([Figure 11-1](#)). It is surrounded by a thin rim of cytoplasm containing some mitochondria, free ribosomes, and a small Golgi apparatus ([Figure 11-2](#)). Scanning electron microscopy shows that some lymphocytes are smooth surfaced, whereas others are covered by many small projections ([Figure 11-3](#)). NK cells are usually larger than T cells or B cells and contain obvious cytoplasmic granules. With this exception, lymphocyte structure provides no clue as to their function or complexity ([Figure 11-4](#)).

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FIGURE 11-1 Photomicrographs showing lymphocytes in blood smears from horse, cat, and dog. Giemsa stain. (Courtesy Dr. M.C. Johnson.)

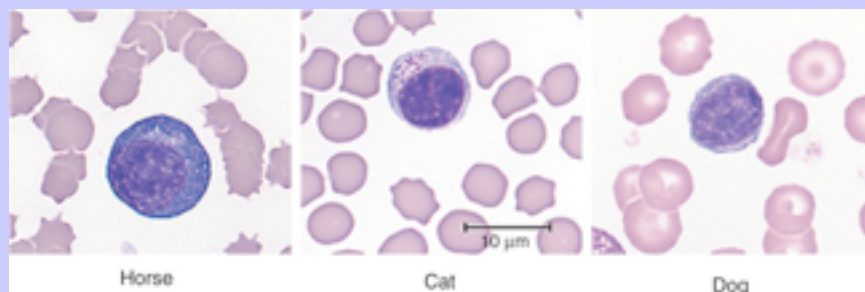


FIGURE 11-2 A transmission electron micrograph of a blood lymphocyte from a rabbit. (Courtesy Dr. S. Linthicum.)

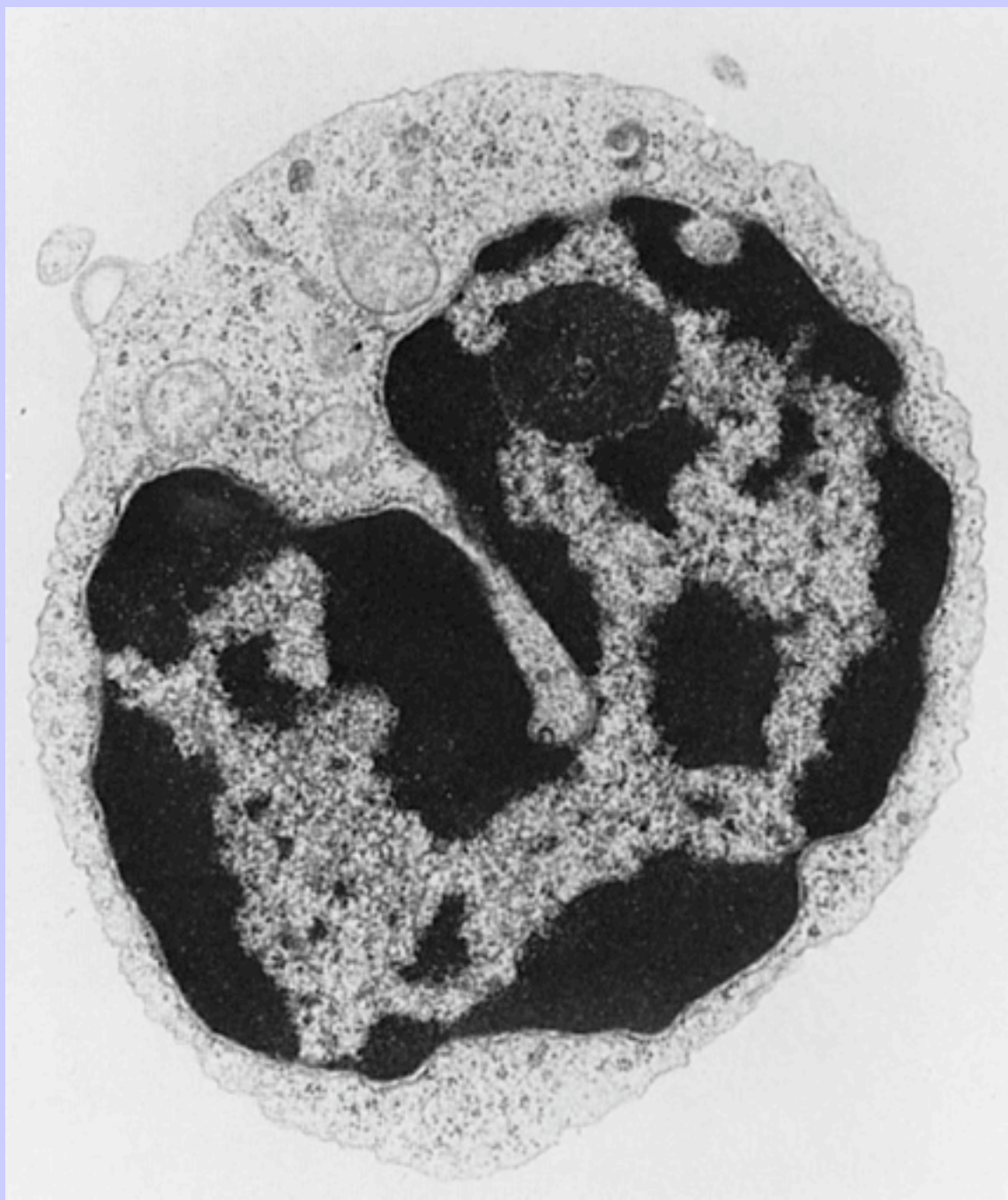
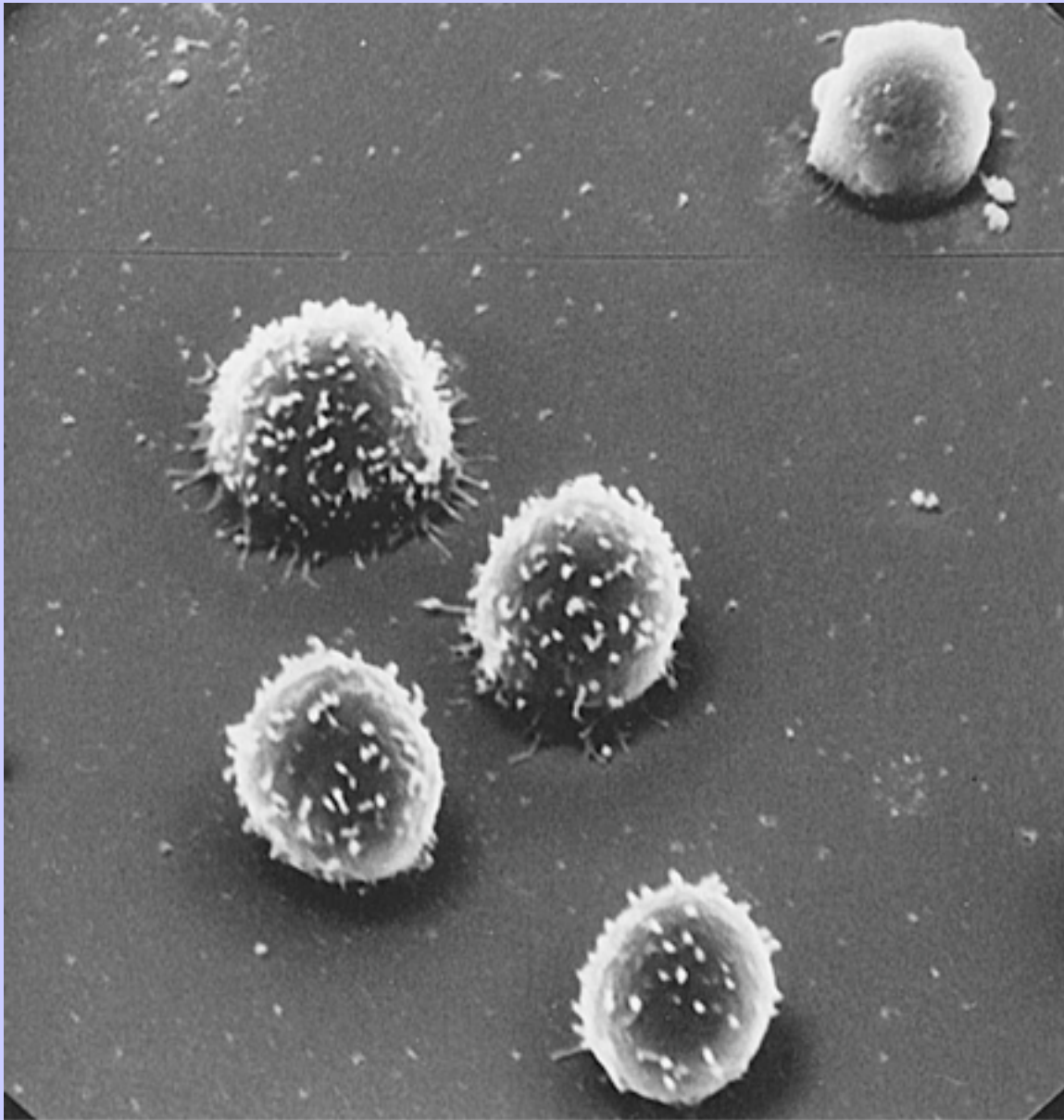


FIGURE 11-3 Scanning electron micrograph of lymphocytes from a mouse lymph node. Original magnification $\times 1500$.



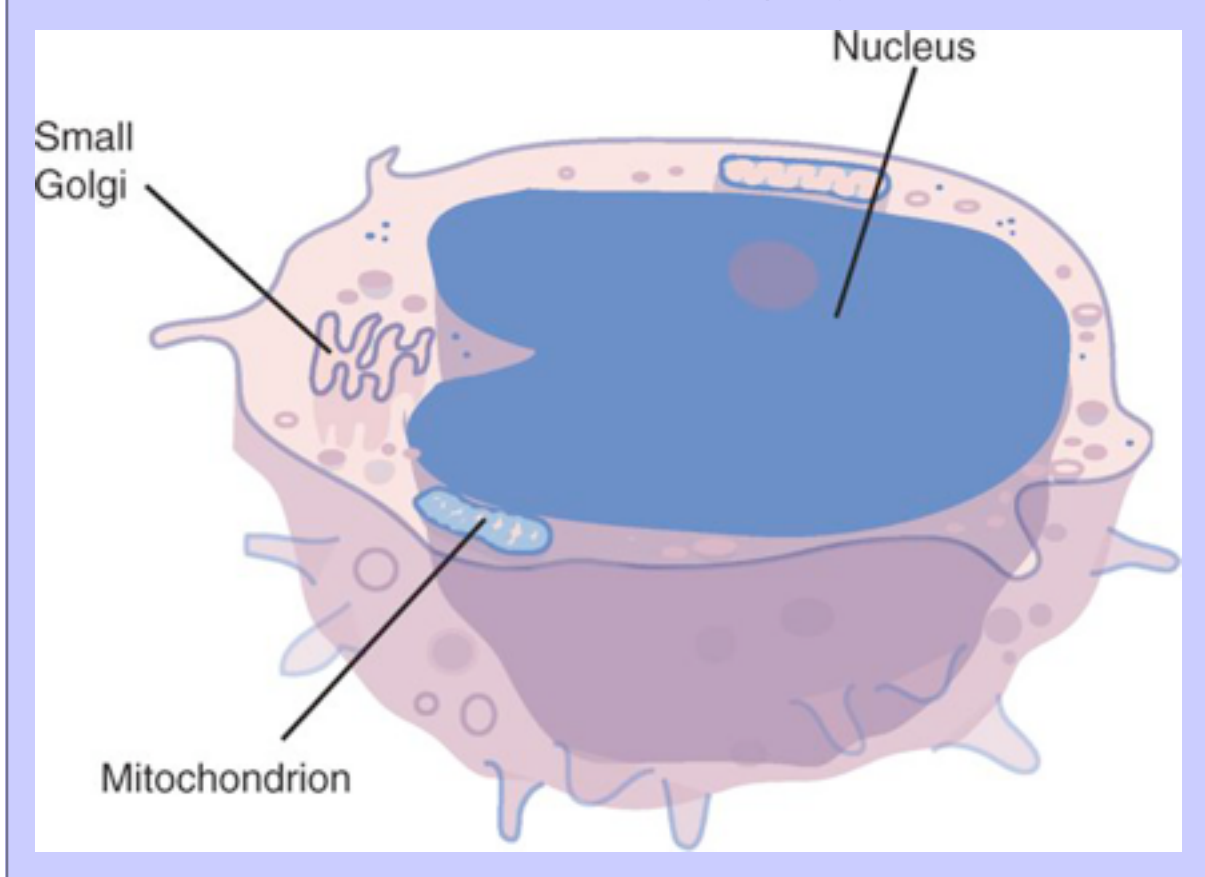
11.3 LYMPHOCYTE POPULATIONS

Lymphocytes are found throughout the body in lymphoid organs, in blood, and scattered under mucosal surfaces ([Figure 11-5](#)). Despite their uniform appearance, they are a diverse mixture of subpopulations. Although these subpopulations cannot be identified by their structure, they can be identified by their characteristic cell surface molecules and by their behavior ([Table 11-1](#)). The pattern of cell surface molecules expressed on a cell is called its

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immunophenotype. By analyzing cell immunophenotypes it is possible to identify many lymphocyte subpopulations.

FIGURE 11-4 Essential structural features of a lymphocyte.



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The loss of cell-mediated immunity as a result of neonatal thymectomy demonstrated the existence of T lymphocytes ([Figure 11-6](#)). After T cells leave the thymus, they accumulate in the paracortex of lymph nodes, the periarteriolar lymphoid sheaths of the spleen, and the interfollicular areas of the Peyer's patches. T cells also account for 60% to 80% of the lymphocytes in blood ([Table 11-2](#)).

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Similar experiments involving bursectomy in chickens pointed to the existence of B cells. In mammals, B cells originate in the bone marrow but mature within Peyer's patches or in the bone marrow before migrating to the secondary lymphoid organs. B cells predominate in the cortex of lymph nodes, in follicles within the Peyer's patches and spleen, and in the marginal zone of the white pulp of the spleen. B cells account for 10% to 40% of blood lymphocytes (see [Table 11-2](#)).

NK cells were identified as a result of the presence of detectable cytotoxic activity in unsensitized animals. NK cells probably originate from the same stem cells as T cells but do not undergo thymic processing. They are widely distributed throughout the lymphoid organs. They account for 5% to 10% of blood lymphocytes.

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11.4 LYMPHOCYTE SURFACE MOLECULES

Many lymphocyte surface molecules have been characterized, especially in humans and mice. Each molecule usually has a functional or chemical name as well as a cluster of differentiation (CD) designation ([Figures 11-7](#) and [11-8](#)). Currently, the CD nomenclature system gives sequential numbers to each molecule: CD4, CD8, CD16, and so on, up to CD350. Since arbitrary numbers are difficult to remember, the basic principle used in this text is that if the molecule's common name is well accepted or describes its function, then that name will be used. Examples include FcαR (CD89), interleukin-6R (CD126), and L-selectin (CD62L). CD nomenclature will also be used for molecules where the designation is well accepted, such as CD8 and CD4. It will also be used for molecules that have an irrational or arbitrary abbreviation or acronym. A list of relevant CD molecules and their functions can be found in [Appendix 1](#).

CD molecules expressed on the cells of the domestic mammals fall into two categories. The great majority of them are also found in humans and mice (homologs) and so have the same CD number. There are, however, several cell surface molecules found in these species that have no recognized homolog in man or mouse. These unattributable molecules have been given a species abbreviation and the prefix WC (work

FIGURE 11-5 Location of lymphocytes in the body.

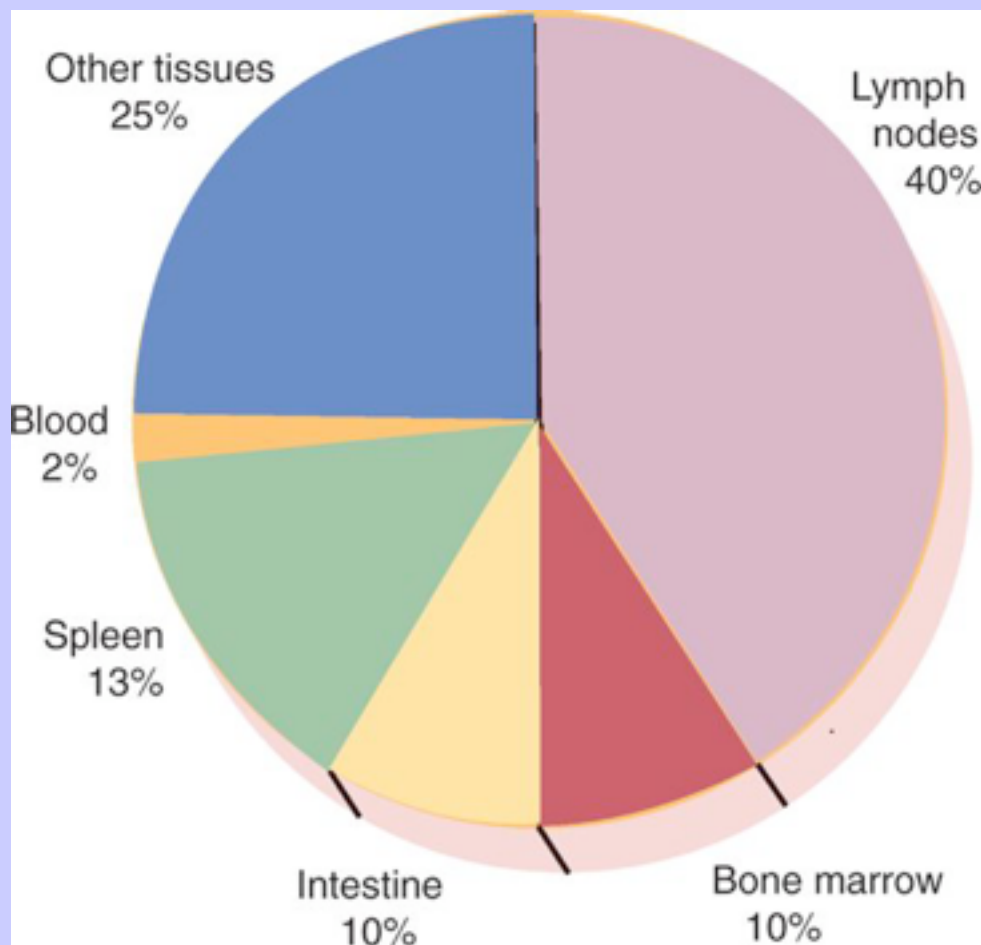
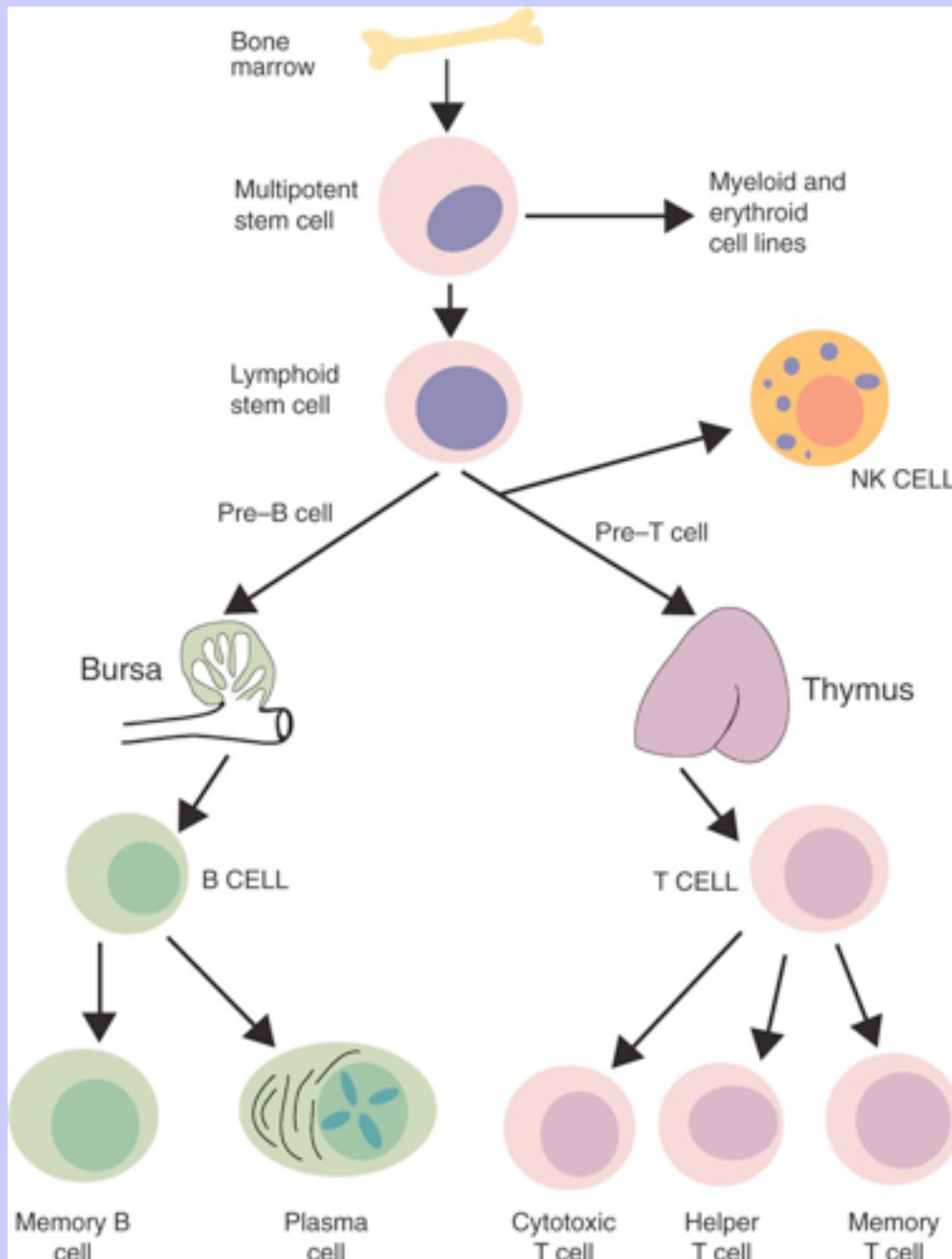


FIGURE 11-6 Development of T and B lymphocytes. Both arise from bone marrow precursors. B cells develop in the bursa, Peyer's patches, or bone marrow. T cells develop in the thymus. Natural killer (NK) cells are a third population of lymphocytes that are distinct from T cells and B cells.



shop cluster), such as BoWC1 and BoWC2 found in cattle.

Table 11-1 Identifying Features of T and B Cells

Property	B Cells	T Cells
Develop within	Bone marrow, bursa, Peyer's patches	Thymus
Distribution	Lymph node cortex Splenic follicles	Lymph node paracortex Spleen periarteriolar sheath
Circulate	No	Yes
Antigen receptors	BCR-immunoglobulin	TCR-protein heterodimer Associated with CD3, CD4, or CD8
Important surface antigens	Immunoglobulins	CD2, CD3, CD4, or CD8
Mitogens	Pokeweed, lipopolysaccharide	Phytohemagglutinin, concanavalin A, BCG vaccine, pokeweed
Antigens recognized	Free foreign proteins	Processed foreign proteins in MHC antigens
Tolerance induction	Difficult	Easy
Progeny cells	Plasma cells, memory cells	Effector T cells, memory T cells
Secreted products	Immunoglobulins	Cytokines

11.4.1 The Antigen Receptor Complex

The most important structures on the surface of lymphocytes are their antigen receptors. These are abbreviated TCR (T cell antigen receptor) or BCR (B cell antigen receptor). Both are complex structures containing many different proteins. Some of these proteins bind antigen, whereas others are used for signal transduction. There are two major populations of T cells differentiated by their TCR. One uses paired α and β peptide chains (TCR α/β), and the other uses paired γ and δ chains (TCR γ/δ). There are also subpopulations of B cells that use five different peptide chains (γ , μ , α , ϵ , and δ) in their BCR. BCRs also differ from TCRs in that they are shed in large amounts into tissue fluid and the blood, where they are called antibodies. Thus antibodies are simply soluble BCRs.

NK cells do not have antigen receptors like T cells and B cells. They, in contrast, have receptors for surface molecules expressed on healthy normal cells but absent from diseased, abnormal cells. NK cells kill target cells that fail to express these surface molecules.

CD3 is the collective name given to the proteins in the TCR that transmit a signal from the receptor to the T cell once antigen is bound. CD3 is therefore found on all T cells. Another protein, CD4, is found only on the T cells that can recognize processed exogenous antigens, the T helper cells. CD4 is a receptor for major histocompatibility complex (MHC) class II molecules on antigen-presenting cells. CD8, in contrast, is only expressed on T cells that attack and kill abnormal cells, the cytotoxic T cells. CD8 is a receptor for MHC class I molecules. Most human and mouse T cells express either CD4 or CD8, rarely both. For example, about 65% of

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human T cells are CD4⁺CD8⁻ and 30% are CD4⁻CD8⁺. The remaining T cells express neither (CD4⁻CD8⁻) and are said to be double negative. The ratio of CD4⁺ to CD8⁺ cells in blood may be used to estimate lymphocyte function. An elevated CD4 count implies increased lymphocyte reactivity because helper cells predominate, whereas a high CD8 count implies depressed lymphocyte reactivity. The relative proportions of CD4 and CD8 cells differ between humans and other mammals ([Table 11-3](#)). Neither CD4 nor CD8 is expressed on B cells or NK cells.

Table 11-2 Major Peripheral Blood Lymphocyte Populations in Mammals as Percentages of the Total Population

	T Cells	B Cells	CD4 ⁺	CD8 ⁺	CD4/CD8
Horses	38–66	17–38 [*]	56 [†]	20–37 [‡]	4.75 [‡]
Bovine	45–53 [§]	16–21 [§]	8–31	10–30	1.53 [§]
Sheep	56–64 [¶]	11–50 [¶]	8–22 [¶]	4–22 [¶]	1.55 [¶]
Pigs	45–57 [#]	13–38 ^{**}	23–43	17–39	1.4 ^{††}
Dogs	46–72	7–30	27–33 [‡]	17–18 [‡]	1.7 [‡]
ssCats	31–89 ^{‡‡}	6–50 ^{‡‡}	19–49 ^{‡‡}	6–39 ^{‡‡}	1.9 ^{‡‡}
Human	70–75	10–15	43–48 ^{§§}	22–24 ^{§§}	1.9–2.4 ^{§§}

* McGorum BC et al: *Vet Immunol Immunopathol* 36:207-222, 1993.

† Grunig G et al: *Vet Immunol Immunopathol* 42:61-69, 1994.

‡ Rivas AL et al: *Vet Immunol Immunopathol* 45:55-71, 1995.

§ Park YH et al: *J Dairy Sci* 75:998-1006, 1992.

¶ Thorp BH et al: *Dev Comp Immunol* 15:393-400, 1991.

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Pescovitz MD et al: *Vet Immunol Immunopathol* 43:53-62, 1994

** Saalmüller A et al: *Vet Immunol Immunopathol* 43:45-52, 1994.

†† Joling P et al: *Vet Immunol Immunopathol* 40:105-118, 1994.

‡‡ Walker R et al: *Aust Vet J* 72:93-97, 1995.

§§ Bleavins MR et al: *Vet Immunol Immunopathol* 37:1-13, 1993.

FIGURE 11-7 Major surface receptors of B cells, their ligands, and their functions.

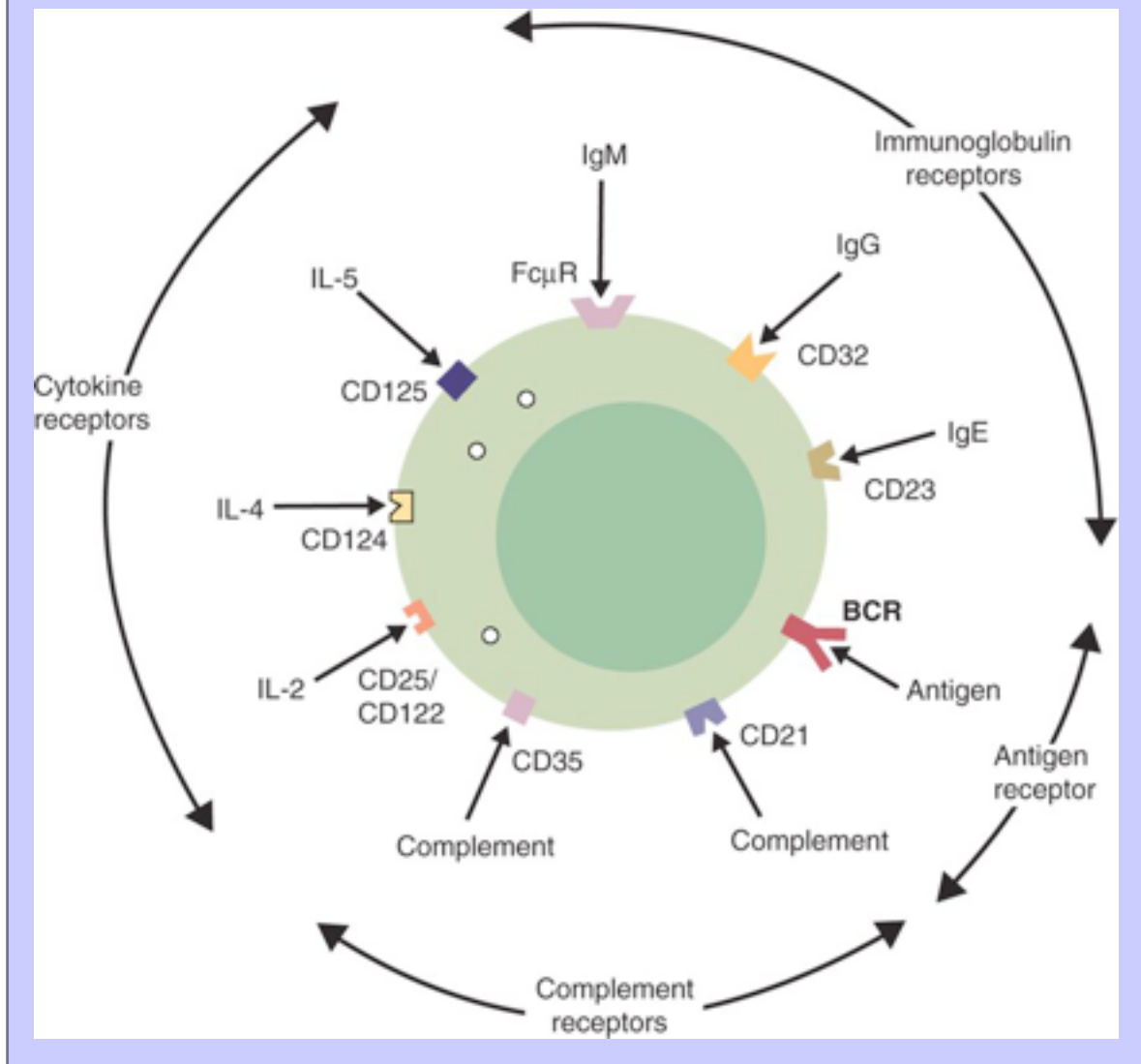


FIGURE 11-8 Major surface receptors of T cells, their ligands, and their functions.

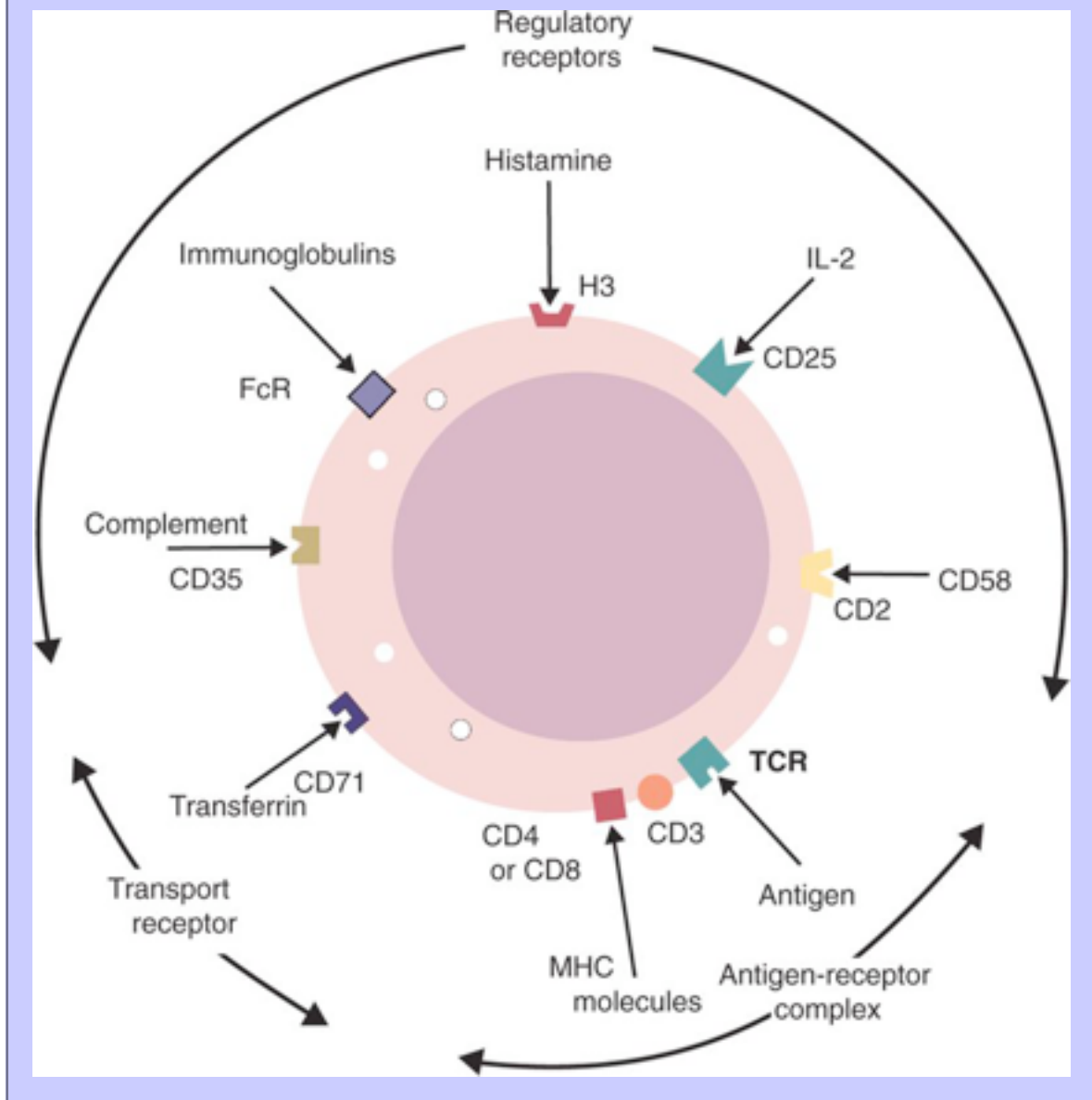


Table 11-3 Surface Molecules on Peripheral Blood T Cells

Marker	Cell Percentage			
	Mouse	Bovine	Swine	Sheep
TCR- α/β	85–95	5–30	14–34	5–30
TCR- γ/δ	5–15	45–50	31–39	22–68
CD2	95	41–60	58–72	10–36
CD4	24	8–28	23–43	8–22
CD8	11	10–30	17–39	4–22
WC1	—	5–44	40	15–70

CD45 is expressed on all three lymphocyte populations. For example, about 10% of the T cell surface is covered by CD45 molecules. These regulate signal transduction by the TCR. Different forms of CD45 have been identified. Thus naïve T cells express one form of CD45, whereas stimulated and memory T cells express another.

The signal-transducing components of the BCR complex are two small heterodimers formed by pairing CD79a (immunoglobulin- α [Ig- α]) with CD79b (Ig- β). These are discussed in detail in [Chapter 13](#).

11.4.2 Molecules That Regulate Lymphocyte Function

Proteins on cell surfaces serve physiological functions. Some are enzymes, some are transport proteins, and many are receptors. All cells use receptor molecules to communicate with their environment. They need receptors to bind other cells as well as receptors for cytokines, for antibodies, and for complement components.

11.4.2.1 Cytokine Receptors

Lymphocytes have many different cytokine receptors. Examples include CD25, which is part of the interleukin-2 (IL-2) receptor; CD118, an interferon receptor; CD120, the tumor necrosis factor (TNF) receptor; and CDw210, the IL-10 receptor. (These are discussed in [Chapter 6](#).)

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Table 11-4 Receptors for IgG (FcγR)

Property	FcγRI	FcγRII	FcγRIII
CD designation	CD64	CD32	CD16
Molecular weight	75 kDa	39-48 kDa	50-65 kDa
Cells	Monocytes, macrophages	B cells, macrophages, granulocytes, eosinophils	NK cells, granulocytes, macrophages
Affinity	High	Moderate	Low
Function	Phagocytosis	B cells: inhibition Macrophages: phagocytosis	NK cells: ADCC Granulocytes: phagocytosis

11.4.2.2

Antibody Receptors

Lymphocytes have receptors for antibodies. Since these receptors bind to the Fc regions of antibody molecules, they are called Fc receptors (FcRs). (The meaning of the term *Fc* can be found in [Chapter 13](#).) The FcRs for IgG are designated FcγR since they bind the γ chain of IgG. Likewise those for IgA are designated FcαR and those for IgE are FcεR. Receptors for IgM have been identified on both B cells and T cells but are not well characterized.

Three different IgG receptors have been described on leukocytes ([Table 11-4](#)). They are called FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16). All are multichain glycoproteins. One chain usually binds the anti-body, whereas the other chains are needed for signal transduction. Sometimes these signal-transducing chains are shared with other receptors such as the TCR.

CD64 (FcγRI) is found on dendritic cells, monocytes, and macrophages and to a much lesser extent on neutrophils. (It is not found on lymphocytes but is mentioned here for the sake of completeness.) CD64 binds IgG with high affinity.

CD32 (FcγRII) is found on B cells, dendritic cells, and macrophages. It has a moderate affinity for IgG and will therefore only bind immune complexes (several antibody molecules attached to an antigen). There are three subtypes of CD32 called a, b, and c. The function of CD32c is unclear. CD32b is found on B cells, where it is an inhibitory receptor and regulates antibody production. CD32a is expressed on macrophages and neutrophils, where it is an activating receptor. It promotes phagocytosis and stimulates the release of cytokines. All three subtypes are expressed on dendritic cells and stimulate dendritic cell maturation and antigen presentation.

CD16 (FcγRIII) binds IgG with low affinity and will therefore only bind immune complexes. It is found on granulocytes, NK cells, and macrophages but not on B cells. Signaling through CD16 can trigger NK cell activation.

Cattle have a unique FcR called Fcγ2R. It is not related to other mammalian FcγRs but belongs to a novel gene family that includes FcαRI (CD89). It is expressed on myeloid cells and binds only aggregated bovine IgG2. It may be important in promoting phagocytosis and antibody-dependent cell-mediated cyto-toxicity.

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FcαRI (CD89) is expressed on neutrophils, eosinophils, monocytes/macrophages, and dendritic cells. It binds IgA and mediates its endocytosis and recycling.

FcεRI is a high-affinity IgE receptor found on mast cells (see [Chapter 25](#)). It plays an important role in allergies. CD23, or FcγRII, in contrast, is a low-affinity IgE receptor expressed on activated B cells, platelets, eosinophils, macrophages, NK cells, dendritic cells, and possibly even T cells. Activated B cells can secrete soluble CD23, which then regulates allergic responses.

Mice have an additional receptor for IgG called FcγRIV; related proteins are found in humans, chimpanzees, rats, dogs, cats, pigs, and cattle. This receptor binds IgG2a and IgG2b with moderate affinity, but it does not bind IgG1 or IgG3. It is expressed exclusively on neutrophils, macrophages, and dendritic cells.

PIgR and FcRn are FcRs involved in immunoglobulin transport across epithelial surfaces. They are described in [Chapters 18](#) and [19](#).

11.4.2.3

Complement Receptors

There are four major receptors for complement components found on lymphocytes (CR1-4). B cells and activated T cells express CR1 (CD35), which binds C3b and C4b, and CR2 (CD21), which binds C3d and C3bi. CR2 is closely associated with the BCR and regulates B cell responses to antigen. NK cells express CR3 and CR4.

11.4.3

Adherence Molecules

As discussed in [Chapter 3](#), some cell surface molecules bind cells together. They regulate signaling between the cells of the immune system and control the movement of leukocytes in tissues. The cell adhesion molecules found on lymphocytes include integrins, selectins, and members of the immunoglobulin superfamily.

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11.4.3.1

Integrins

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Integrins are heterodimeric proteins classified according to their β chain. For example, the β_1 integrins consist of a β_1 chain (CD29) paired with one of several different α chains (CD49). The β_1 integrins bind cells to extracellular matrix proteins such as fibronectin, laminin, and collagen.

The β_2 integrins consist of a β_2 chain (CD18) paired with one of several α chains (CD11). These β_2 integrins control the binding of leukocytes to vascular endothelium and bind T cells to antigen-presenting cells. For example, leukocyte function-associated antigen-1 (CD11a/CD18) on a T cell binds to its ligand, intercellular adhesion molecule-1 (ICAM-1), on the antigen-presenting cell. By prolonging and stabilizing cell interactions, this binding permits successful antigen recognition ([Figure 11-9](#)).

11.4.3.2

Selectins

The emigration of lymphocytes from the bloodstream into tissues is regulated by P-selectin (CD62P), L-selectin (CD62L), and E-selectin (CD62E). P-selectins and E-selectins are found on vascular endothelial cells. When these cells are activated by inflammation, they express selectins that bind neutrophils, activated T cells,

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and monocytes. L-selectin binds lymphocytes to high-endothelial venules in lymphoid organs (see [Chapter 10](#)).

11.4.3.3 Immunoglobulin Superfamily

Some members of the immunoglobulin superfamily (IgSF) are lymphocyte adhesion molecules. For example, ICAM-1 (CD54) binds to CD11a/CD18 and CD43 (see [Chapter 3](#), [Figure 3-7](#)). ICAM-1 is normally expressed on dendritic cells and B cells. Inflammation induces ICAM-1 expression on vascular endothelial cells and so permits phagocytic cells to bind and move into inflamed tissues. ICAM-1 is also responsible for the migration of T cells into areas of inflammation (so-called delayed hypersensitivity reactions, discussed in [Chapter 28](#)). Another IgSF adherence molecule is vascular cell adhesion molecule-1 (VCAM-1), or CD106. VCAM-1 is expressed on inflamed vascular endothelial cells. It binds the β_1 integrin, CD49d/CD29, found on lymphocytes and monocytes.

11.4.3.4 CD58 and CD2

CD58 is the ligand for CD2. CD2 is found only on T cells, whereas CD58 is widely distributed on many cell types. CD2 and CD58 bind when cytotoxic T cells encounter their target cells, and it is likely that CD58 facilitates T cell binding to any cell that is to undergo surveillance (see [Chapter 16](#)). CD58 is also found on antigen-presenting cells such as dendritic cells and macrophages. When it binds T cell CD2, CD58 enhances the recognition of antigen by the T cell and at the same time stimulates the antigen-presenting cell to secrete cytokines.

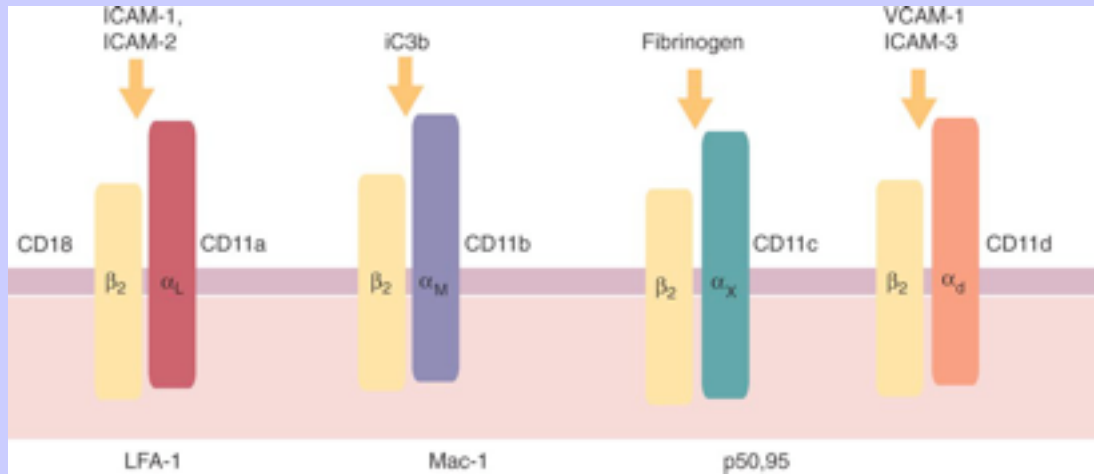
11.4.4 Other Major Surface Molecules

B cells function as antigen-presenting cells and express MHC class II molecules on their surface. In contrast, T cell expression of MHC class II varies between species. Both types of lymphocytes express MHC class Ia and class Ib molecules.

11.4.4.1 CD1

CD1 molecules are a family of nonpolymorphic MHC molecules that present lipid and glycolipid antigens to

FIGURE 11-9 The integrin families are based on the pairing of many different chains with a limited number of β chains. This example shows the structure of the very important β_2 integrins that act as cell surface adhesion molecules to link cells together so that they can communicate privately.



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T cells. There are two separate groups of CD1 molecules. Some are expressed on antigen-presenting cells, especially dendritic cells and B cells. Others are expressed on intestinal epithelium and some thymocytes. In mice and humans CD1d presents glycolipids to a T cell subset called NKT cells. Cattle have two CD1d pseudogenes but no functional genes, so they may also lack NKT cells.

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11.4.4.2

WC1

Lymphocytes of the major domestic mammals have several cell surface proteins not found in either humans or mice. The best defined of these is WC1, a single chain type 1 glycoprotein with a mass of 220 kDa found on about 90% of bovine γ/δ T cells. In cattle WC1⁺ T cells are found in high numbers in the skin and mucous membranes, as well as in hemal nodes and the thymus. WC1 belongs to a protein family found in many different mammals, amphibians, and invertebrates. WC1 cDNA probes hybridize with human, rodent, and horse DNA, suggesting that related genes exist in these species but are not expressed. Multiple copies of the WC1 gene are found in cattle and sheep, and homologs of WC1 have been identified in camels, llamas, deer, and elk. Although its natural ligand is unknown, WC1 probably binds to ligands on macrophages and dendritic cells. It may have similar functions to CD4 and CD8.

11.4.5

Changes in Immunophenotype

Lymphocytes do not express the same immunophenotype at all stages in their life cycle. A cell's phenotype depends on its maturity and activation status. For example, immature human T cells carry both CD9 and CD10. As the T cells mature within the thymus, CD9 is lost and the cells gain CD4 and CD8. Mature thymocytes can then split into two subpopulations: one population becomes CD4⁺CD8⁻, the other becomes CD4⁻CD8⁺. In

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addition, the phenotype of lymphocytes changes after exposure to antigen. Thus naïve T cells express high levels of CD45R and L-selectin and low levels of CD44. Memory T cells show the reverse of this—low levels of CD45R and L-selectin and high levels of CD44.

11.5 SPECIES DIFFERENCES

11.5.1 Horses

Horse lymphocytes express four species-specific proteins, EqWC1 through EqWC4. EqWC1 is found on 70% of equine T cells, 30% of B cells, and 50% of granulocytes. It may be a homolog of CD90. EqWC2 is found on granulocytes and most T cells. (Additional studies sometimes show that a WC molecule may in fact have a CD homolog. For example, EqWC3 is CD2 and EqWC4 may be a homolog of CD28.)

11.5.2 Bovine

Cattle lymphocytes express the species-specific cell surface molecules BoWC1 through BoWC15. (BoWC3 is now known to be CD21 and BoWC10 is CD26.)

In adult cattle about 10% to 15% of circulating T cells are γ/δ positive, whereas the remainder are α/β positive. In young calves the proportion of γ/δ T cells may rise to 40%. However, this proportion fluctuates in response to management and stress. The majority of bovine γ/δ T cells also express WC1. Indeed, these cells can be activated either through their TCR or through WC1. In response they produce TNF- α , IL-1, IL-12, and interferon- γ . This suggests that they can promote inflammation while contributing to a Th1 bias in the bovine immune response and so link the innate and acquired immune systems.

CD4 is expressed on 20% to 30% of blood lymphocytes in adult ruminants. Double-negative T cells constitute 15% to 30% of the blood lymphocytes in young ruminants, but this may reach 80% in newborn calves. Most of these double-negative cells are γ/δ^+ and WC1 $^+$. Thus the major circulating T cells in ruminants (γ/δ^+ , WC1 $^+$, CD4 $^-$, CD8 $^-$) are different from the predominant T cells in humans and mice (α/β^+ , WC1 $^-$, CD4 $^+$, CD8 $^-$).

11.5.3 Sheep

Sheep T cells express OvWC1 (also called T19). The isoform of this molecule expressed on α/β T cells is different from the molecule expressed on γ/δ T cells. When lambs are born, γ/δ T cells account for 60% of blood T cells, but this drops to 30% by 1 year of age and to 5% by 5 years.

11.5.4 Pigs

Pig leukocytes express nine unique surface proteins (SWC1-SWC9). SWC1 is expressed on resting T cells, monocytes, and granulocytes but not on B cells. SWC3 is found on monocytes/macrophages. SWC9 is expressed only by mature macrophages. In young pigs 66% of blood T cells are γ/δ positive, but this drops to 25% to 50% in adults. Pigs have two subpopulations of γ/δ T cells. One is CD2 $^+$ and the other is CD2 $^-$ and has not been identified in other species. Some pig γ/δ T cells can function as antigen-presenting cells using MHC class II molecules. Up to 60% of T cells in pig blood are double positive CD4 $^+$ CD8 $^+$. The rest are predominantly double negative (CD4 $^-$ CD8 $^-$).

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11.5.5 Dogs and Cats

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In dogs, CD4 is expressed on neutrophils and macrophages but not on monocytes, whereas in cats CD4 is found on only a subset of T cells and their precursors.

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11.6 LYMPHOCYTE MITOGENS

In addition to their surface proteins, lymphocytes can be characterized by the stimulants that make them divide. The most important of these are proteins, called lectins, that bind to cell surface glycoproteins and so trigger cell division ([Box 11-1](#)). These lectins are commonly isolated from plants. Examples include phytohemagglutinin (PHA) obtained from the red kidney bean (*Phaseolus vulgaris*), concanavalin A (Con A) obtained from the jack bean (*Canavalia ensiformis*), and pokeweed mitogen (PWM) obtained from the pokeweed plant (*Phytolacca americana*). Lectins specifically bind sugar residues on glycoprotein side chains. For example, PHA binds *N*-acetylgalactosamine, and Con A binds α -mannose and α -glucose. Not all lymphocytes respond equally well to all lectins. Thus PHA primarily stimulates T cells, although it has a slight effect on B cells. Con A is also a T cell mitogen, whereas PWM acts on both T and B cells.

Although the plant lectins are the most efficient lymphocyte mitogens, mitogens are also found in other unexpected sources. For example, an extract from the snail *Helix pomata* stimulates T cells whereas lipopolysaccharide from Gram-negative bacteria stimulates B cells. Other important B cell mitogens include proteases, such as trypsin, and Fc fragments of immunoglobulins. Bacille Calmette-Guérin vaccine, an avirulent strain of *Mycobacterium bovis* that is used as a vaccine against tuberculosis, is a T cell mitogen. These mitogens can assist in the differentiation of T cells and B cells and, by measurement of the response provoked, provide an estimate of their responsiveness.

11.6.1 Box 11-1 How to Measure Mitogenicity

To measure the effect of mitogens, lymphocytes are grown in tissue culture. Lymphocytes can be obtained directly from blood. The lymphocytes are cultured for at least 24 hours before the mitogen is added. Once this is done they begin to divide, synthesize new DNA, and take up any available nucleotides from the medium. It is usual to incorporate a small quantity of thymidine labeled with the radioactive isotope of hydrogen, tritium (^3H), in the tissue culture fluid. The thymidine is only incorporated into the DNA of cells that are dividing. After about 24 hours, the cultured cells are separated from the tissue culture fluid, either by centrifugation or filtration, and their radioactivity is counted. The amount of radioactivity in the mitogen-treated cells may be compared with that in an untreated lymphocyte culture. This ratio is called the stimulation index. As an alternative to the use of tritiated thymidine, a radiolabeled amino acid such as ^{14}C -leucine can be used. Uptake of this compound indicates increased protein synthesis by the cells.

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¹² CHAPTER 12 Helper T Cells and Their Response to Antigen

^{12.1} KEY POINTS

- Helper T cells possess antigen receptors (TCRs) consisting of two peptide chains, either α and β or γ and δ .
- These receptors have an antigen-binding groove that can bind to antigenic peptides linked to a major histocompatibility complex (MHC) molecule on an antigen-presenting cell.
- The antigen-binding chains of the TCR are linked to a complex signal-transducing component called CD3.
- Each TCR is associated with either CD4 or CD8. CD4 binds to MHC class II molecules on the antigen-presenting cell. CD8 binds to MHC class I molecules expressed on all nucleated cells.
- In order to respond to antigens, T cells must bind to antigenic peptides linked to the MHC. They must also receive co-stimulation from cytokines and other co-stimulatory molecules.
- The signals from an antigen-presenting cell are communicated to a T cell through an immunological synapse.
- There are three major subpopulations of helper T cells. Th1 cells are stimulated by interleukin-12 (IL-12) and secrete IL-2 and interferon- γ in response. They generally promote cell-mediated responses.
- Th2 cells are stimulated by IL-1 and secrete IL-4, IL-13, and IL-10. They generally promote antibody responses.
- Th17 cell development is stimulated by IL-6, transforming growth factor- β , and IL-23. They secrete IL-17 and promote neutrophil-mediated inflammation.
- α/β T helper cells are the predominant T cells in most mammals. γ/δ Helper cells are mainly confined to the intestinal wall in humans but are the predominant circulating T cells in young ruminants.

Unlike the innate immune responses that are triggered by a limited number of molecular patterns restricted to the major groups of pathogenic microorganisms, the lymphocytes of the acquired immune system are able to recognize and respond to “everything,” or at least to a large number of very diverse foreign antigens. These lymphocytes have receptors that bind specific antigens and, under the right conditions, mount cell-mediated or antibody-mediated immune responses.

There are three major populations of lymphocytes with antigen-binding receptors. These are the helper T cells that regulate immune responses, the effector or cytotoxic T cells that destroy cells expressing endogenous antigens, and the B cells that produce antibodies to destroy exogenous antigens. The cells in each of these populations are selected so that only antigens that bind to their specific receptors can trigger an immune response. This chapter discusses the first of these lymphocyte populations, the helper T cells.

Exogenous antigen trapped and processed by dendritic and other antigen-presenting cells is presented to helper T cells in secondary lymphoid organs. Each T cell is covered by its own unique set of identical antigen receptors. If these receptors bind antigen in the correct manner, the helper T cell is triggered to initiate an immune response by secreting cytokines, dividing, and differentiating. As will be discussed later, the other antigen-responsive cell populations, the B cells and the cytotoxic T cells, cannot respond optimally to antigens unless they too are stimulated by helper T cells.

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Because of the central role of helper T cells, they must be carefully regulated through cell-cell interactions and by the activities of many different cytokines.

It is important to point out at this stage that the antigen receptors on T cells do not develop in order to bind to specific foreign antigens. On the contrary, these antigen receptors are generated randomly. As a result, the antigen receptors on all the T cells in the body form a large, diverse repertoire. It may be expected that any foreign antigen that enters the body will encounter and bind to at least one T cell. Because each T cell has only one receptor specificity, the repertoire of receptors is, in effect, the repertoire of the T cells. Given the random nature of receptor binding, the strength of binding (or affinity) between an antigen and its receptors will vary. Thus an antigen may be bound strongly by some receptors and weakly by others. If binding strength is very weak, the encounter between the antigen and its receptor may be insufficient to activate the T cell.

In a newborn animal that has never encountered antigens previously, the number of T cells that can bind any specific antigen may be very low. In order to increase the probability of an antigen encountering a T cell with the correct receptor, the T cells are concentrated in organs such as lymph nodes, where their chances of a successful interaction with antigen-bearing dendritic cells are maximized. In primed animals, where mature T cells are plentiful, they can migrate into the tissues where they can encounter other antigen-presenting cells, such as macrophages and B cells. T cell antigen receptors have evolved to recognize the complex formed between an antigenic peptide and a major histocompatibility complex (MHC) molecule. They cannot recognize or respond to free antigen molecules.

12.2 THE IMMUNOGLOBULIN SUPERFAMILY

Proteins are constructed by the linkage of multiple peptide modules or domains. Each domain usually has a specialized function. For example, in proteins located on a cell surface, the membrane-binding domain contains hydrophobic amino acids that allow it to penetrate the plasma membrane. Other domains may be responsible for the structural stability of a protein or for its biological activities. In antibody (immunoglobulin) molecules, one domain is used to bind antigen and other domains are responsible for cell binding. The presence of similar domains in dissimilar proteins suggests that they have a common origin and proteins may be assigned to families or superfamilies based on their domain structure.

Proteins belonging to the immunoglobulin superfamily play key roles in the immune system. The members of this superfamily all contain at least one immunoglobulin domain. In a typical immunoglobulin domain the peptide chains weave back and forth to form a pleated sheet that folds into a sandwich-like structure. Immunoglobulin domains were first identified in antibody molecules (immunoglobulins). They have since been found in many other proteins, and collectively these proteins form the immunoglobulin superfamily. The superfamily includes some proteins with multiple immunoglobulin domains and some with only a single domain. Important proteins with multiple domains include the B cell antigen receptors (BCRs), the T cell antigen receptors (TCRs), and the MHC class I and II molecules ([Figure 12-1](#)). All of the members of this superfamily are receptors; most are found on cell surfaces; and none has enzymatic activity. Many cellular interactions are mediated by binding between two different members of the superfamily, as, for example, between TCR and MHC molecules.

12.3 THE T CELL ANTIGEN RECEPTOR

12.3.1 The Antigen-Binding Component

Each T cell has about 30,000 antigen receptors (TCRs) on its surface. All the TCRs expressed on an individual cell are identical, so, in effect, a T cell can only respond to the peptide that corresponds to its receptors. Each

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TCR is a complex structure containing multiple glycoprotein chains. Two of these chains are paired to form the antigen-binding component; the other chains transmit the signal generated by antigen binding to the cell. Two different types of TCR have been identified based on the peptide chains used for antigen binding ([Figure 12-2](#)). One type employs chains called γ and δ (γ/δ). The other employs two different chains called α and β (α/β). In humans, mice, and probably most nonruminants, 90% to 99% of T cells use α/β receptors. In calves and lambs, in contrast, as many as 60% of T cells may use γ/δ receptors ([Box 12-1](#)).

The four antigen-binding chains (α , β , γ , δ) are similar in structure, although they differ in size. Thus

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FIGURE 12-1 The four key antigen receptors of the immune system—T cell antigen receptor (*TCR*), major histocompatibility complex (*MHC*) class I, MHC class II, and B cell antigen receptor (*BCR*)—are constructed using immunoglobulin domains as building blocks. Each binds antigen through the use of variable domains. All are members of the immunoglobulin superfamily.

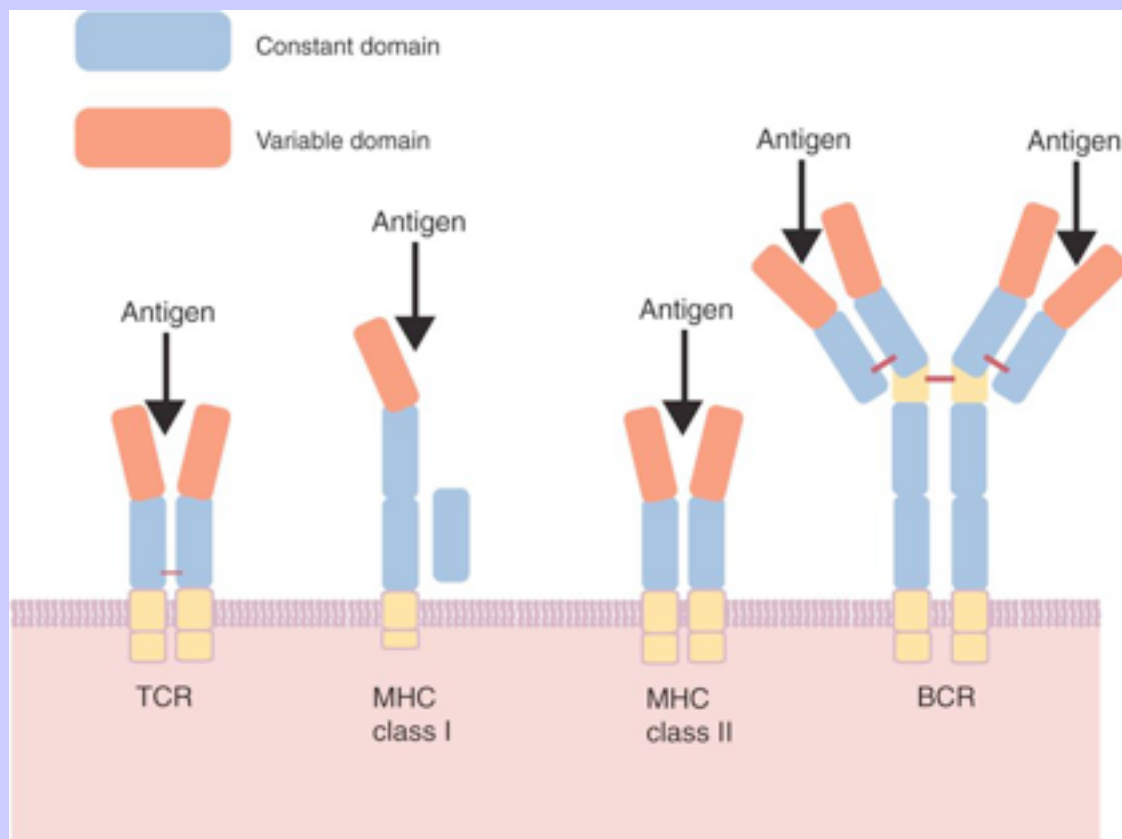
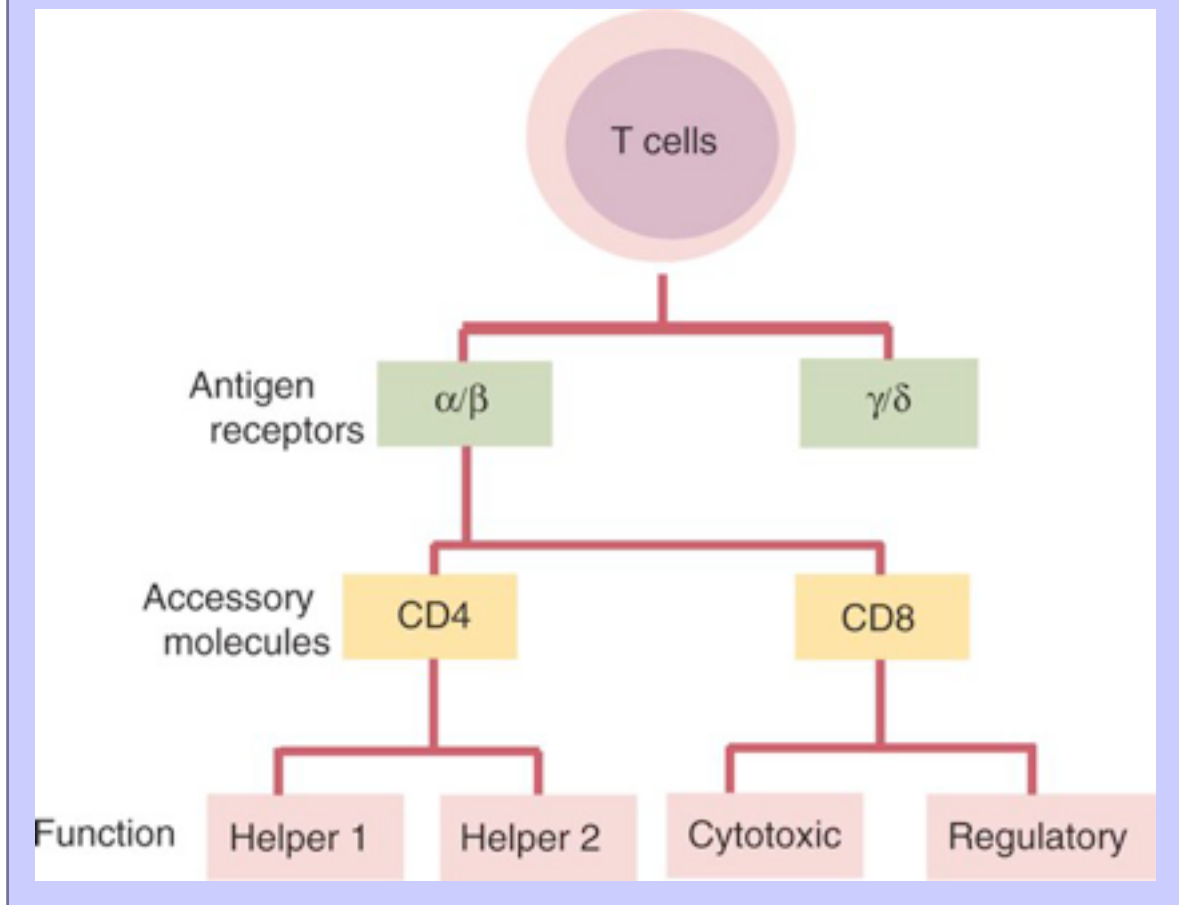


FIGURE 12-2 T cells can be divided into many different subpopulations based on the antigen receptors they employ, on the accessory molecules that support their activity, and ultimately on their functions.



the α chain is 43 to 49 kDa, the β chain is 38 to 44 kDa, the γ chain is 36 to 46 kDa, and the δ chain is 40 kDa. The ranges are due to variations in glycosylation. Each TCR chain is formed from four domains ([Figure 12-3](#)). The N-terminal domain contains about 100 amino acids whose sequence varies greatly among cells. This is therefore called the variable (V) domain. The second domain contains about 150 amino acids. Its amino acid sequence does not vary, so it is called the constant (C) domain. The third, very small domain consists of 20 hydrophobic amino acids passing through the T cell membrane. The C-terminal domain within the cytoplasm of the T cell is only 5 to 15 amino acids long. The two chains are joined by a disulfide bond between their constant domains to form a stable heterodimer.

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FIGURE 12-3 Schematic diagram showing the domain structure of the two peptide chains that make up the antigen-binding component of an α/β T cell antigen receptor.

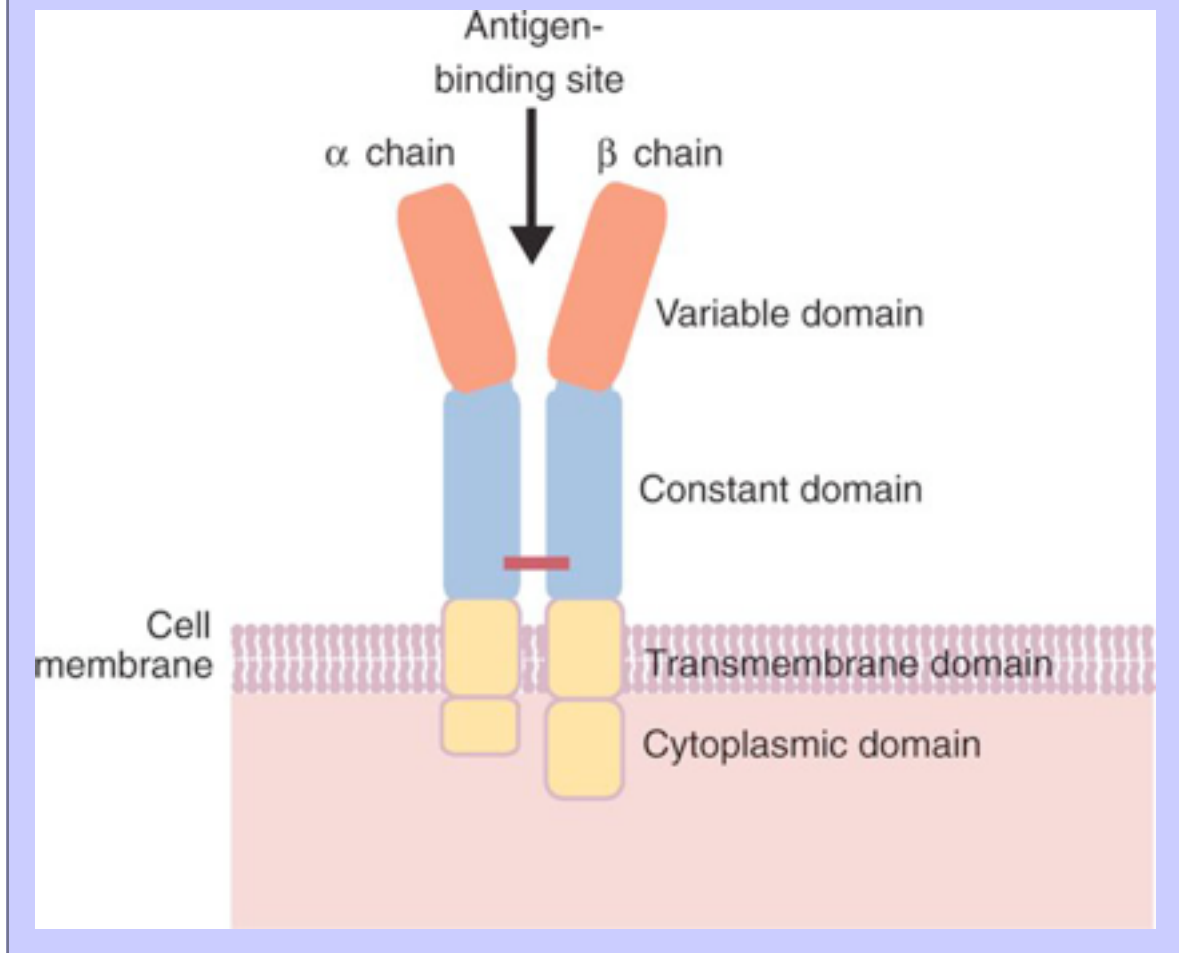
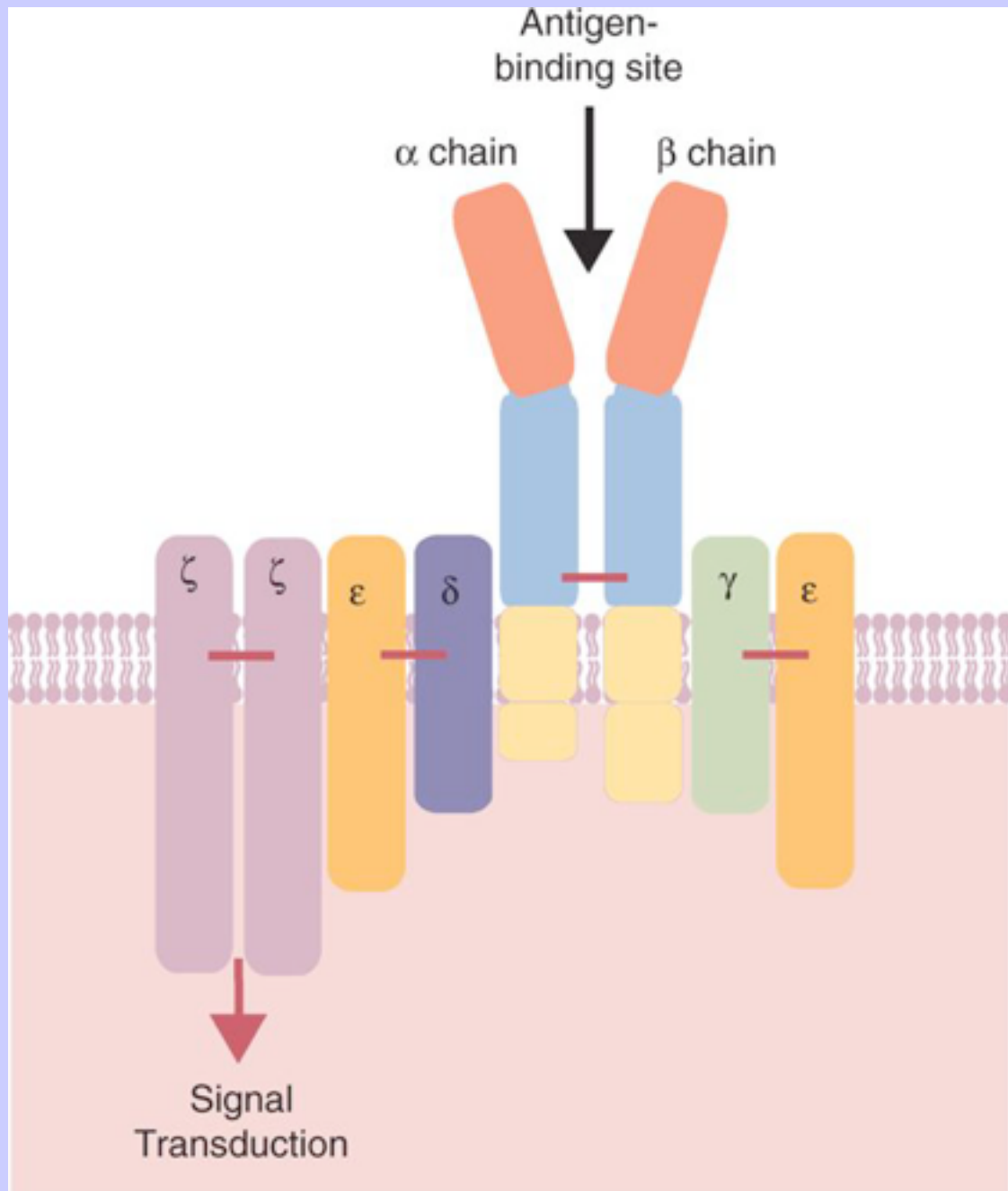


FIGURE 12-4 Overall structure of the T cell antigen receptor (TCR) complex. The signal transduction proteins are collectively classified as CD3. Approximately 80% of α/β TCRs use $\zeta\zeta$ dimers. The remaining 20% use $\eta\zeta$ heterodimers. Most γ/δ TCRs probably use a completely different signal transduction complex.



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Because each TCR consists of paired chains, the two V domains form a groove in which antigens and MHC molecules bind. The precise shape of this antigen-binding groove varies among different TCRs because of the variable amino acid sequences in the V domains. The specificity of the binding between a TCR and an antigen is determined by the shape of the groove formed by the V domains.

Table 12-1 The TCR-CD3 Receptor Complex

Peptide Chain	Function	Molecular Weight (kDa)
TCR α	Recognition of antigen and MHC	45–60
TCR β		40–55
TCR γ	Recognition of antigen	36–46
TCR δ		40–60
CD3 γ	Signal transducer	21–28
CD3 δ	Signal transducer	20–28
CD3 ϵ	Signal transducer	20–25
CD3 ζ	Signal transducer	16
CD3 η	Signal transducer	22
CD4	MHC II receptor	55
CD8	MHC I receptor	34

12.3.1.1 Box 12-1 Are Neutrophils T Cells?

A remarkable study* showed that a subpopulation of human and mice neutrophils express functional T cell receptors (TCRs). They constitute 5% to 8% of human neutrophils. In addition to expressing α/β receptors, these cells possess a recombinase activating gene 1 (RAG-1)/RAG-2 complex. Engagement of the neutrophil TCR complex protects the cells from apoptosis and stimulates their secretion of interleukin-8.

This finding is very surprising. Previously, neutrophils had been considered short-lived phagocytic cells engaged exclusively in inflammation. T cells in contrast are antigen-specific lymphocytes engaged exclusively in acquired immunity. These cell types have not been considered to be functionally linked. It will be essential to confirm these findings and to determine if such cells exist in the domestic mammals.

* Puellmann K, Kaminski WE, Vogel M, et al: A variable immuno-receptor in a population of human neutrophils, *Proc Natl Acad Sci* 103:14441-14446, 2006.

When the V domains are examined closely, it is found that within each V domain is an area of the chain where the amino acid sequence is especially highly variable. This is the region that actually comes into con-tact with the antigen. For this reason it is called the hypervariable or the complementarity-determining region (CDR). The antigen-binding site of the TCR is formed by the paired CDRs that line the groove. The rest of the V domain outside the CDRs has a constant sequence and is called the framework region.

12.3.2 The Signal Transduction Component

12.3.2.1 CD3

The binding of antigen to the TCR sends a signal to trigger the T cell response. The two antigen-binding chains of each TCR are associated with a cluster of proteins called the CD3 complex (Figure 12-4). The CD3 complex consists of five protein chains (γ , δ , ϵ , ζ , and η) (Table 12-1) arranged as three dimers γ - ϵ , δ - ϵ , and ζ - ζ or ζ - η . The TCR β chain is linked to the γ - ϵ dimer, and the TCR α chain is linked to the δ - ϵ dimer. About 80% of α/β TCRs contain a ζ - ζ homodimer, so that the complete complex consists of $\alpha\beta$ - $\gamma\epsilon$ - $\delta\epsilon$ - $\zeta\zeta$. The remaining 20% contain ζ - η heterodimers. (They therefore consist of $\alpha\beta$ - $\gamma\epsilon$ - $\delta\epsilon$ - $\zeta\eta$.)

12.3.2.2 CD4 and CD8

Two other proteins closely associated with the TCR are CD4 and CD8. CD4 is a single-chain glycoprotein of 55 kDa, and CD8 is a dimer of 68 kDa. (One chain is called a, the other is b. In humans, pigs, mice, and cats, CD8 is an α - β heterodimer or, less commonly, an α - α homodimer.) Both CD4 and CD8 are members of the

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FIGURE 12-5 Role of CD4 and CD8 in promoting T cell responses. These molecules link the T cell to the antigen-presenting cell, binding the two cells together and ensuring that an effective signal is transmitted between them. CD4 binds to major histocompatibility complex (MHC) class I molecules. This interaction is seen in Chapter 8, Figure 8-6, A.

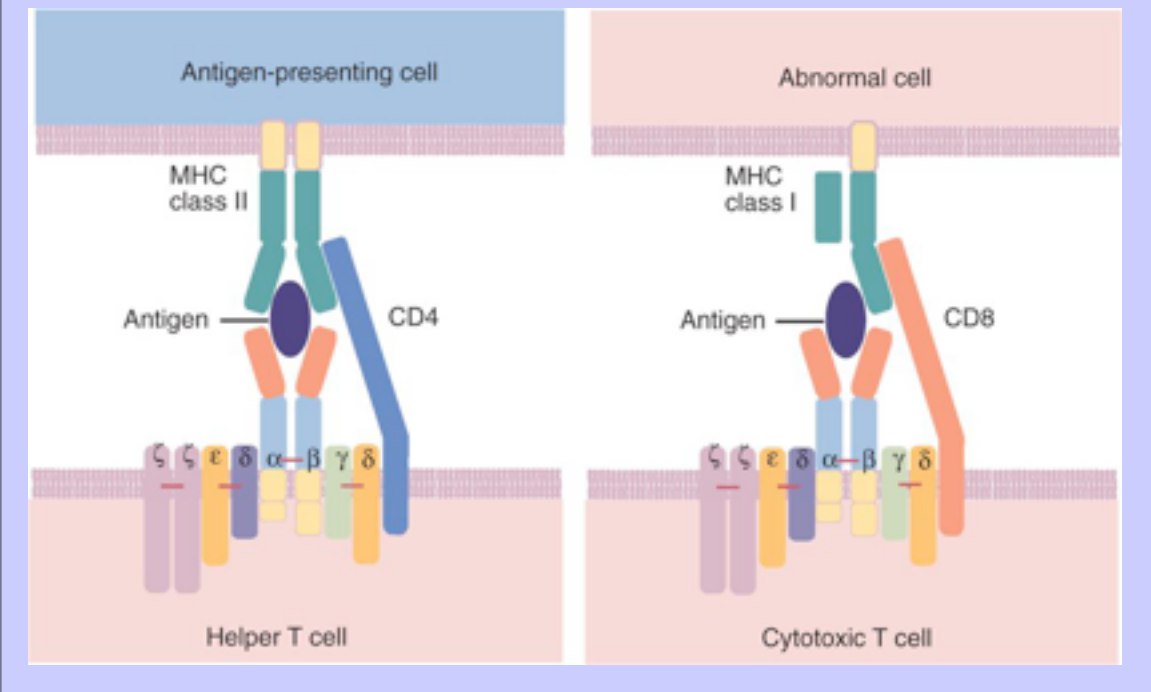
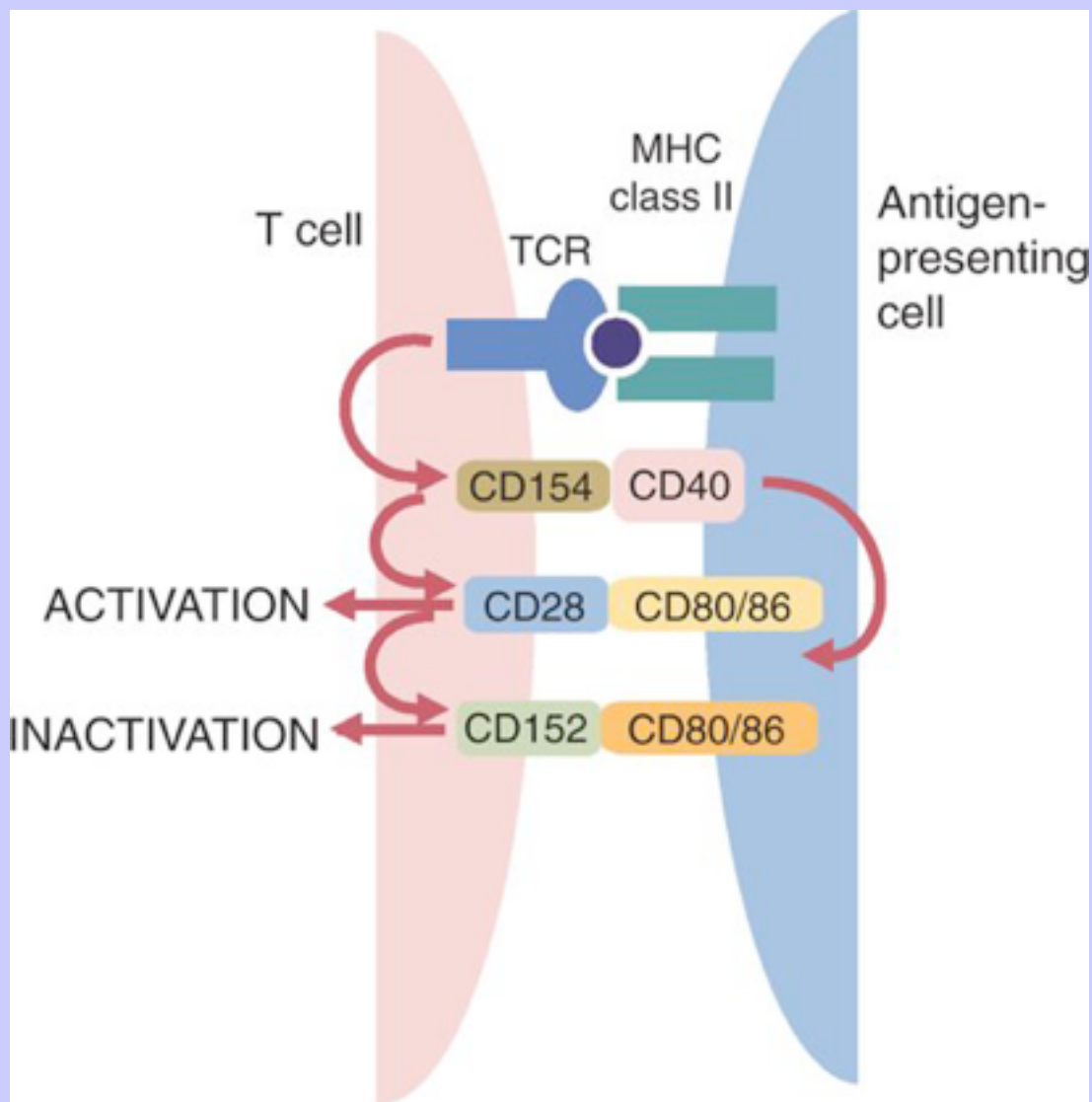


FIGURE 12-6 Antigen-presenting cells and helper T cells engage in a dialog. Thus binding of antigen to the T cell antigen receptor (*TCR*) causes the T cell to express CD40 ligand (*CD154*). This engages CD40 on the antigen-presenting cell. As a result, CD28 and CD152 are expressed on the T cell and CD80 or CD86 is expressed on the antigen-presenting cell. Depending on which receptors are engaged, the T cell may be stimulated or suppressed.



immunoglobulin superfamily. The presence of CD4 or CD8 determines the class of MHC molecule that is recognized by the T cell ([Figure 12-5](#)). For example, CD4, found only on helper T cells, binds MHC class II

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molecules on antigen-presenting cells. CD8, in contrast, is found only on cytotoxic T cells and binds MHC class Ia molecules. CD4 and CD8 enhance TCR signal transduction 100-fold when they bind to an MHC molecule on an antigen-presenting cell.

12.4 CO-STIMULATORS

The binding of a TCR to a peptide-MHC complex is not sufficient by itself to trigger a helper T cell response. Additional signals are needed. These co-stimulatory signals are of three types. First, ligands such as CD40 on antigen-presenting cells bind receptors on T cells. Their role is to amplify responses once initial T cell activation is achieved. Second, T cells are stimulated by cytokines secreted by the antigen-presenting cells. These determine the way in which a T cell responds to antigen. Finally, for maximal effect, adhesion molecules must bind the T cells and antigen-presenting cells firmly together and so permit prolonged, strong signaling between the cells.

12.4.1 Co-Stimulatory Signals

CD40 is a receptor expressed on antigen-presenting cells. Its ligand is CD154 that is expressed on helper T cells several hours after their TCRs have bound antigen ([Figure 12-6](#)). When CD154 and CD40 bind, signals are sent in both directions. The T cell sends signals to the antigen-presenting cell and the antigen-presenting cell signals the T cell. The signal to the T cell causes it to express a receptor called CD28. The signal to the antigen-presenting cell stimulates it to express CD80 or CD86 or both. Binding of CD40 to CD154 also stimulates the antigen-presenting cell to secrete multiple cytokines, including interleukin-1 (IL-1), IL-6, IL-8, IL-12, CCL3, and tumor necrosis factor- α (TNF- α). This signal also prolongs dendritic cell survival, permits B cells to respond to antigen, and activates macrophages. CD28, the T cell receptor induced by CD40 \leftrightarrow CD154 signaling, has two ligands: CD80, found on dendritic cells, macrophages, or activated B cells; and CD86, found on B cells. When CD28 binds to CD80 or CD86 it stimulates the T cell to express yet another receptor, CD152 (CTLA-4). CD152 can also bind to CD80 or CD86. The binding of CD28 to CD80 or CD86 is required for complete helper T cell activation since the engagement of CD28 amplifies the stimulus to the T cell 100-fold. CD28 stimulation enhances the production of IL-2 and other cytokines, it upregulates cell survival genes, it promotes energy metabolism, and it facilitates cell division. On the other hand, when CD152 binds to CD80 or CD86, T cell activation is suppressed. Thus the opposing signals delivered to T cells through these two receptors, CD28 and CD152, regulate the intensity of T cell responses.

Resting antigen-presenting cells express neither CD80 nor CD86. It takes 48 to 72 hours after T cell CD154 binds to CD40 before antigen-presenting cells express CD80/86 and T cells express CD152. Both CD80 and CD86 can bind to either CD28 or CD152. However, because CD152 binds these molecules with a higher affinity than does CD28, the inhibitory effect of CD80/86 gradually predominates. When CD152 binds to CD80 on dendritic cells, it induces the production of indoleamine dioxygenase (IDO), an enzyme that destroys tryptophan. In the absence of this amino acid, T cells cannot respond to antigen and the T cell response is terminated.

12.4.2 Co-Stimulatory Cytokines

Cytokines, as described previously, are short-range signaling proteins that regulate immune cell functions. Cytokine secretion by antigen-presenting cells is triggered by many different stimuli, including micro-bial pathogen-associated molecular patterns (PAMPs) binding to toll-like receptors (TLRs). Cytokine secretion can also be induced by T cells signaling through CD40 and CD154. As described in the chapter on dendritic cells, different dendritic cell populations secrete different cytokine mixtures. These mixtures in turn influence the nature of the helper T cell response. For example, the development of a subpopulation of helper T cells called

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Th1 cells is stimulated by IL-12 from dendritic cells (DC1) or macrophages (M1). IL-12 stimulates Th1 cells to produce interferon- γ (IFN- γ) and IL-2. Complete Th1 cell activation, proliferation, and maximal IFN- γ production are achieved by additional stimulation with IL-18. On the other hand, dendritic cells that secrete IL-1 or IL-4 (DC2 cells) preferentially stimulate a subpopulation of helper T cells called Th2 cells. IL-1, produced by antigen-presenting cells, may either be secreted into the tissue fluid (IL-1 β) or remain bound to the cell surface (IL-1 α), where it will stimulate adherent T cells. Dendritic cells and macrophages stimulated through TLR2 secrete IL-23. This cytokine, together with IL-6 and transforming growth factor- β (TGF- β), stimulates a helper T cell subpopulation called Th17 cells. Th17 cells characteristically secrete IL-17 and IL-21 and promote neutrophil-mediated acute inflammation.

Three related cytokines, all members of the IL-12 family, play a critical role in the development of helper cell populations. IL-12 is a heterodimer consisting of paired p35 and p40 subunits produced mainly by macrophages, dendritic cells, and B cells. It synergizes with TNF- α in promoting IFN- γ production. As a secondary effect, IL-12 reduces immunoglobulin E (IgE) production by suppressing IL-4 synthesis. IL-23 is also a heterodimer that shares the p40 chain with IL-12. The IL-12 and IL-23 receptors also share a β chain. IL-23 promotes the activities of Th17 cells and the secretion of IL-17, leading to neutrophil-mediated inflammation. IL-27 is a third member of the IL-12 family. It shares sequence homology with both IL-12 and IL-23. Originally thought to stimulate Th1 cells, it is now known to be a regulatory cytokine that suppresses the activities of all three helper cell populations.

IL-18 is produced, like IL-1 β , by cleavage of a larger precursor by caspase-1. It too acts on Th1 cells to promote the production of IFN- γ and other cytokines. This can lead to positive feedback, where the IL-18 and IFN- γ reinforce each other's activities.

12.4.3 Adherence Molecules

In addition to the dialog mediated by co-stimulatory molecules, T cells and antigen-presenting cells stimulate each other most effectively if they are held in close contact by adhesive molecules such as the integrins. For example, CD2 and CD11a/CD18 on T cells bind to their ligands CD58 and CD54 on antigen-presenting cells and lock the cells together.

12.5 IMMUNOLOGICAL SYNAPSE FORMATION

All the molecules described above must interact in the correct manner and in the correct order if a T cell is to respond appropriately to an antigen. Thus when a T cell and an antigen-presenting cell come into contact, the cytoskeleton in each cell is rearranged so that the TCR-peptide-MHC complexes and the co-stimulatory receptors cluster together to form a specialized structure in the area of contact called an immunological synapse ([Figure 12-7](#)). This synapse consists of con

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FIGURE 12-7 Interaction between a T cell and an antigen-presenting cell generated the supramolecular structure called an immunological synapse. Thus a series of concentric rings forms around the interacting T cell antigen receptor–major histocompatibility complex. These rings contain different co-stimulating molecules.

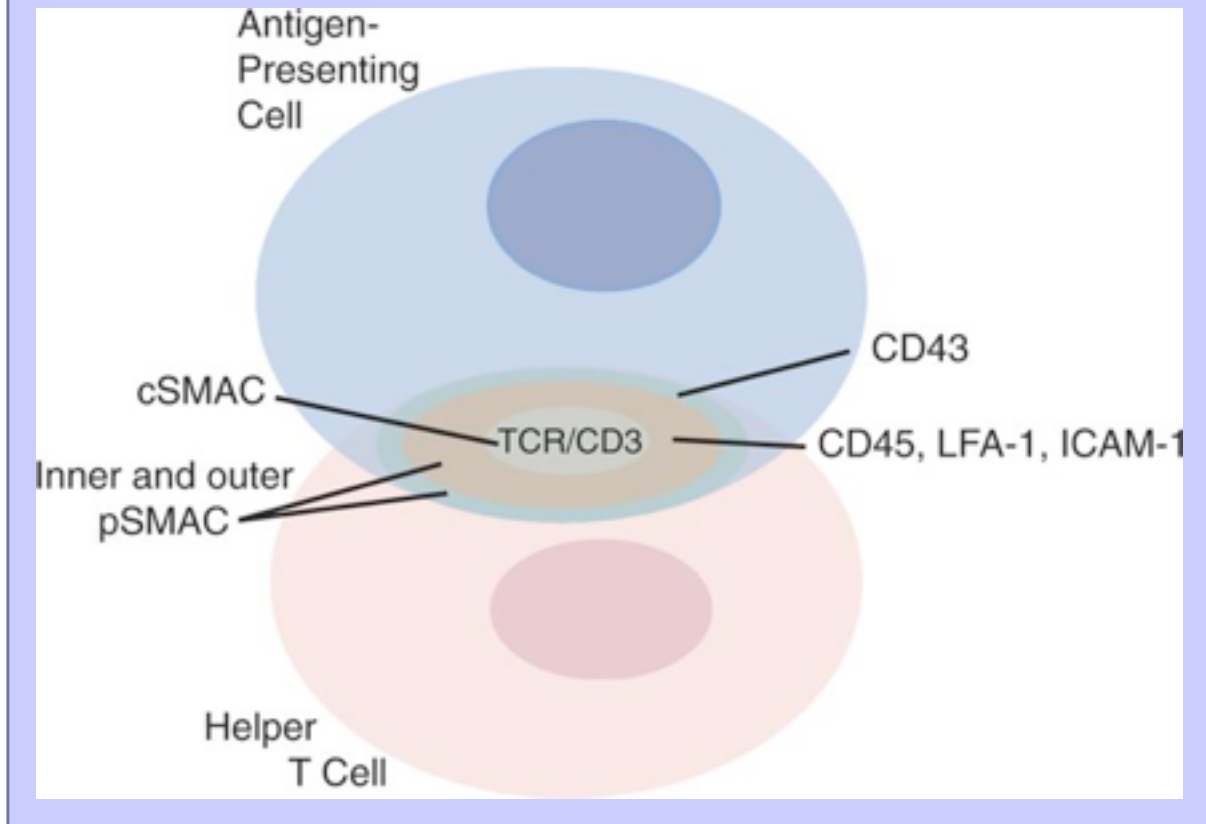


FIGURE 12-8 The time-course of events following the stimulation of a T cell by antigen and interleukin-1. c-fos, A transcription factor. (From Krensky AM: *N Engl J Med* 322:515, 1991.)

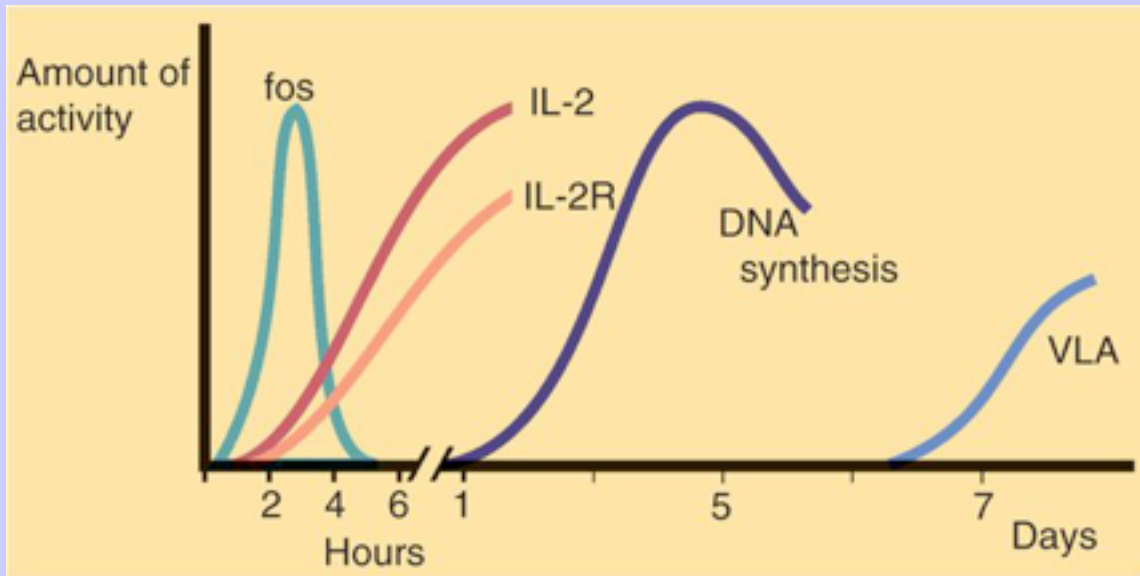
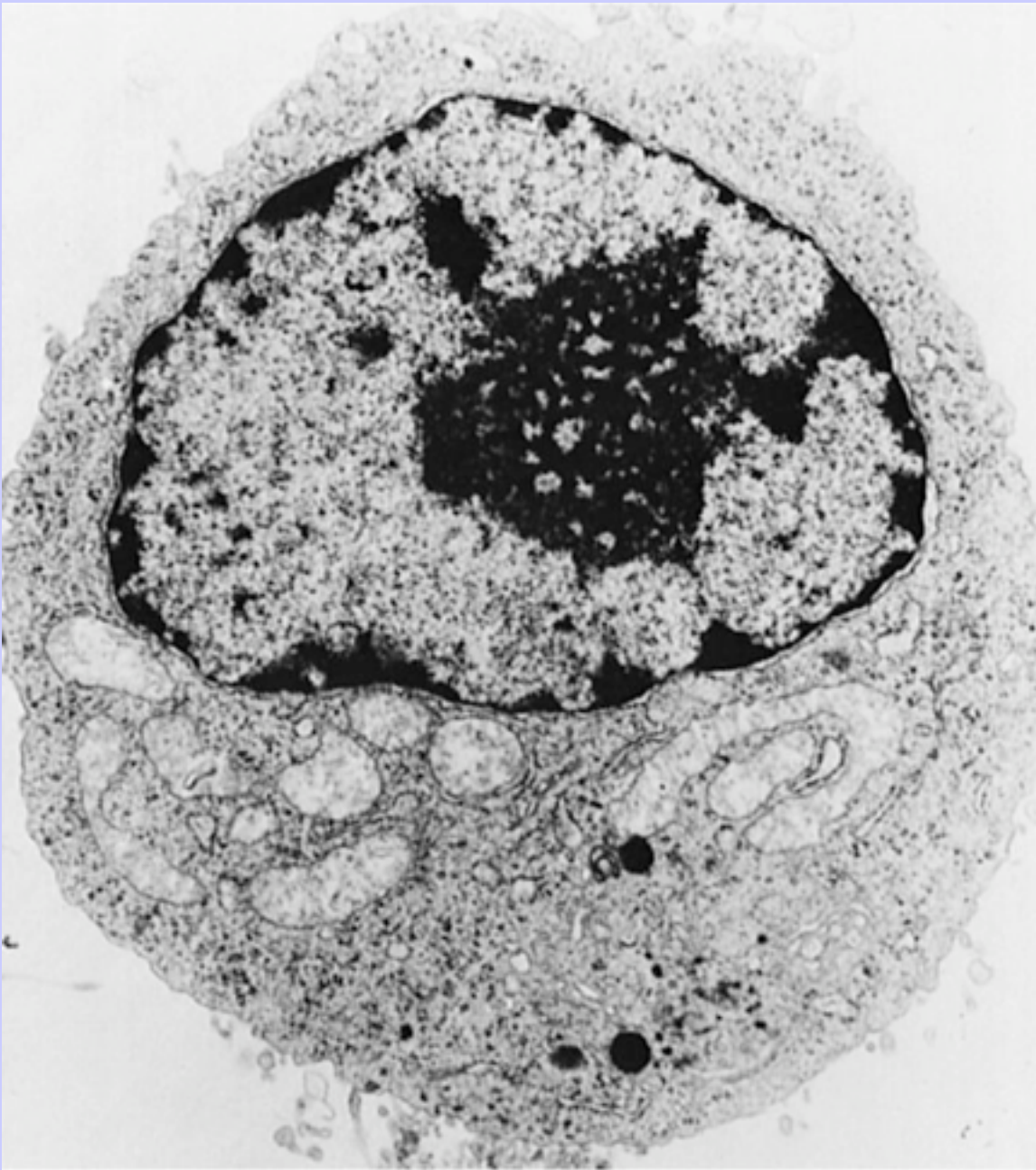


FIGURE 12-9 Transmission electron micrograph of a lymphoblast. Compare this with an unstimulated lymphocyte in [Chapter 11, Figure 11-2](#). Note the extensive cytoplasm, ribosomes, and large mitochondria. (Courtesy Dr. S. Linthicum.)



centric rings of molecular complexes called supra-molecular activation clusters (SMACs). These form a characteristic “bull's eye” consisting of a central (c) SMAC surrounded by a peripheral (p) SMAC and an outer

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ring. The cSMAC contains the MHC and TCR molecules as well as CD4, CD3, CD2, CD28, CD80/86, and CD40/154. The pSMAC contains CD45 and the adhesion molecules intercellular adhesion molecule-1 and leukocyte function-associated antigen-1. A third outer ring contains proteins excluded from the central synapse such as CD43. CD43 is a very large molecule that could interfere with the functioning of the synapse.

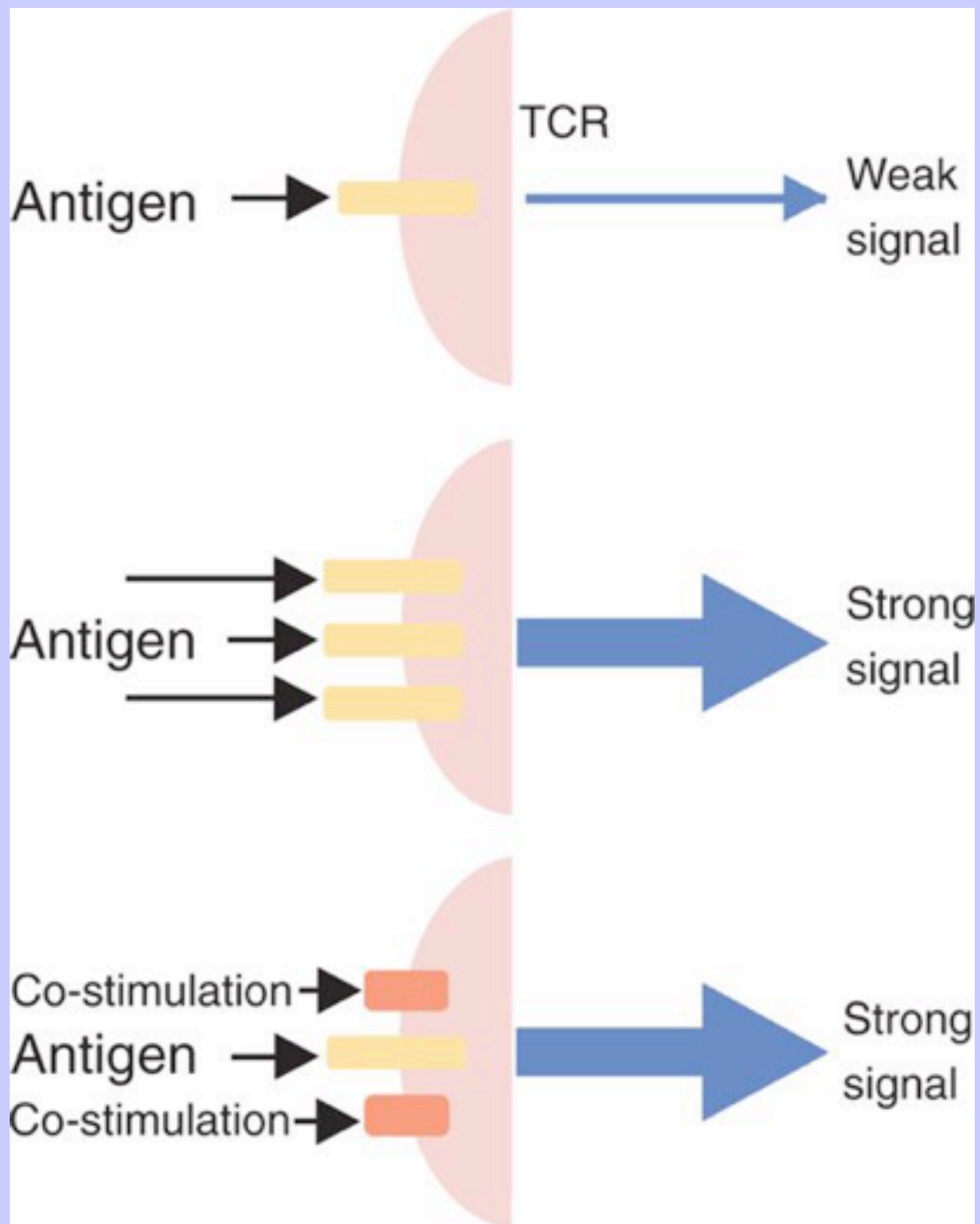
Cell membranes consist of lipid bilayers that are not homogeneous. They contain areas called lipid rafts, where the membrane is enriched in specific lipids and cholesterol. Small rafts are distributed evenly over the surface of resting T cells. When cells interact, these lipid rafts, together with the protein receptors, aggregate to form a synapse. Synapses form within minutes after TCR engagement and are very stable. However, it is important to note that T cells may initially form synapses with multiple antigen-presenting cells but then polarize towards the cell providing the strongest stimulus. Thus, in effect, the T cell seeks the antigen that binds most strongly to its TCR. Once signaling is complete, the components of immunological synapses are endocytosed and degraded, thus terminating cell interactions.

12.6 SIGNAL TRANSDUCTION

Once a TCR binds to antigen on a presenting cell and an immunological synapse forms, the receptor signals to the T cell. The first signal is transmitted from the antigen-binding TCR α and β chains to the CD3 complex ([Figure 12-8](#)). This is probably the result of the clustering of several TCRs together. When the chains are clustered, their immunoreceptor tyrosine-based activation motifs can activate several tyrosine kinases (see [Chapter 6](#)). These phosphorylate a zeta-associated protein-70 (ZAP-70), which triggers three signaling pathways. One pathway activates nuclear factor of activated T cells. The second pathway activates nuclear factor kappa-B. The third pathway generates activator protein-1. Collectively they activate the genes coding for the cytokines, IL-2, IL-2R, IL-3, IL-4, IL-5, IL-6, and IFN- γ ([Figure 12-9](#)). The net effect of

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FIGURE 12-10 Successful stimulation of a T cell requires multiple signals. Depending on the antigen, the T cell may be activated by signals from multiple T cell antigen receptors (*TCR*) or by appropriate co-stimulation.



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these reactions is that T cells enlarge, enter the cell cycle, and synthesize and secrete a mixture of cytokines. The newly produced cytokines trigger the next stages of the immune responses.

12.7 OVERALL CONSIDERATIONS

T cells are highly mobile cells. They migrate rapidly through lymph nodes while continuously scanning the surfaces of dendritic cells for antigens. When a T cell recognizes a foreign antigen, it changes its behavior. It slows down, stops, and eventually binds strongly to the antigen-presenting cell. This contact eventually leads to the formation of an immunological synapse. Whether a T cell stops depends on how strongly it binds to the target antigen. It will not stop for weakly binding antigens.

When an immunological synapse forms, TCRs and co-stimulatory molecules signal to the T cell. However, the TCR does not function simply as a binary (on/off) switch. Instead, differences in the strength of binding, in the amount of co-stimulation, and in the duration of the cell interaction affect T cell responses.

T cell recognition of antigen by helper T cells must be exquisitely sensitive. Because MHC molecules can bind many different antigenic peptides, any individual peptide will usually only be displayed in small amounts. T cells must be able to recognize these few specific peptide-MHC complexes among a vast excess of MHC molecules carrying irrelevant peptides. The number of MHC-peptide complexes signaling to the T cell is also important, since the stimulus needed to trigger a T cell response varies. For example, only one MHC-peptide complex is needed to trigger a CD8⁺ T cell response whereas about 1000 such complexes are required to trigger CD4⁺ T cells. T cell activation, in general, appears to involve tunable thresholds. Each threshold for signaling depends on the level of co-stimulation ([Figure 12-10](#)). For example, a minimum of 8000 TCRs must bind antigen for a CD4⁺ T cell to become activated in the absence of CD28, but only about 1000 TCRs need be engaged if CD28 is present. The duration of signaling also determines a T cell's response. Sustained signaling is required for T cell activation and is maintained by serial triggering of its TCRs. Thus, during the prolonged cell interaction process, each MHC-peptide complex may trigger up to 200 TCRs. This serial triggering depends on the kinetics of TCR-ligand interaction. CD28 increases signal transduction by reducing the time needed to trigger a T cell and lowers the threshold for TCR triggering. Adhesion molecules stabilize the paired T cell and antigen-processing cell and so allow the signal to be sustained for hours. The fate of a helper T cell is determined by the type of antigen-presenting cell used and by the nature of the signal received from it. Thus naïve T cells have strict requirements for activation. They must receive a sustained signal for at least 10 hours in the presence of co-stimulation or for up to 30 hours in its absence. This level of co-stimulation can only be provided by dendritic cells, which supply high levels of co-stimulatory and adhesion molecules. In contrast, other antigen-presenting cells act only transiently. Thus, although macrophages and B cells can briefly trigger a TCR, they are unable to complete the process and so fail to activate naïve T cells. Once primed, T cells require about an hour to reach commitment. Only then can they be activated by macrophages and B cells.

In the absence of effective co-stimulation, a T cell will undergo abortive activation. It does not divide or produce cytokines but either becomes unresponsive to antigen (anergic) or undergoes apoptosis and dies.

12.8 SUPERANTIGENS

When animals are exposed to a foreign antigen, fewer than 1 in 10,000 T cells can bind and respond to an antigen. However, some microbial molecules, called superantigens, are unique in that they may stimulate as many as one in five T cells to divide. It was originally thought that these proteins were simply nonspecific mitogens. This is not the case. Superantigens activate only T cells whose TCR β chains contain certain V domains and to which they can bind. Unlike conventional antigens that must bind within the grooves of both an MHC molecule and a TCR,

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superantigens directly link a TCR V_β domain to an MHC class II molecule on the antigen-presenting cell. All superantigens come from microbial sources, such as streptococci, staphylococci, and mycoplasmas, and from viruses, such as rabies virus. The responses to superantigens are not MHC restricted (i.e., they don't depend on specific MHC haplotypes), but the presence of MHC antigens is required for an effective response since superantigens do not bind to the antigen-binding groove of the MHC class II molecule but attach elsewhere on its surface ([Figure 12-11](#)). As a result, they tightly link the T cell and the antigen-presenting cell together. Because of this strong binding, superantigens trigger a powerful T cell response. This may be a conventional response associated with the secretion of unusually large amounts of cytokines or it may be expressed as tolerance. Indeed, because of the large proportion of T cells stimulated by superantigens, this tolerance can be much less specific than tolerance induced by conventional antigens. Some superantigens may stimulate the secretion of such large amounts of cytokines that they trigger a toxic shock syndrome (see [Chapter 4](#)).

12.9 HELPER T CELL SUBPOPULATIONS

Three major subpopulations of $CD4^+$ helper T cells have been identified. They are called helper 1 (Th1), helper 2 (Th2) T cells, and helper 17 (Th17) cells, and they can be distinguished by the mixture of cytokines that they secrete ([Figure 12-12](#)). As always, many of the details of their function have been investigated in mice and humans, and it must not be assumed that they function in a completely identical manner in other mammals. These major helper cell subpopulations are activated by antigen and co-stimulators presented by different antigen-presenting cells. Thus DC1 cells preferentially stimulate a Th1 response, whereas DC2 cells trigger a Th2 response.

12.9.1 Th1 Cells

Th1 cells respond optimally to antigen presented by myeloid dendritic cells (DC1) and by B cells using the

FIGURE 12-11 Differences in binding to a T cell antigen receptor (TCR) between a conventional antigenic peptide that fills the groove between the α and β chains as opposed to a superantigen that binds only to the β chain.

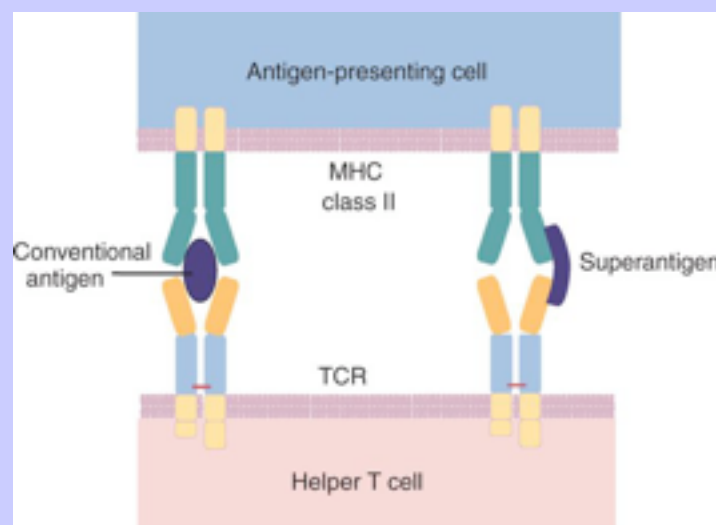
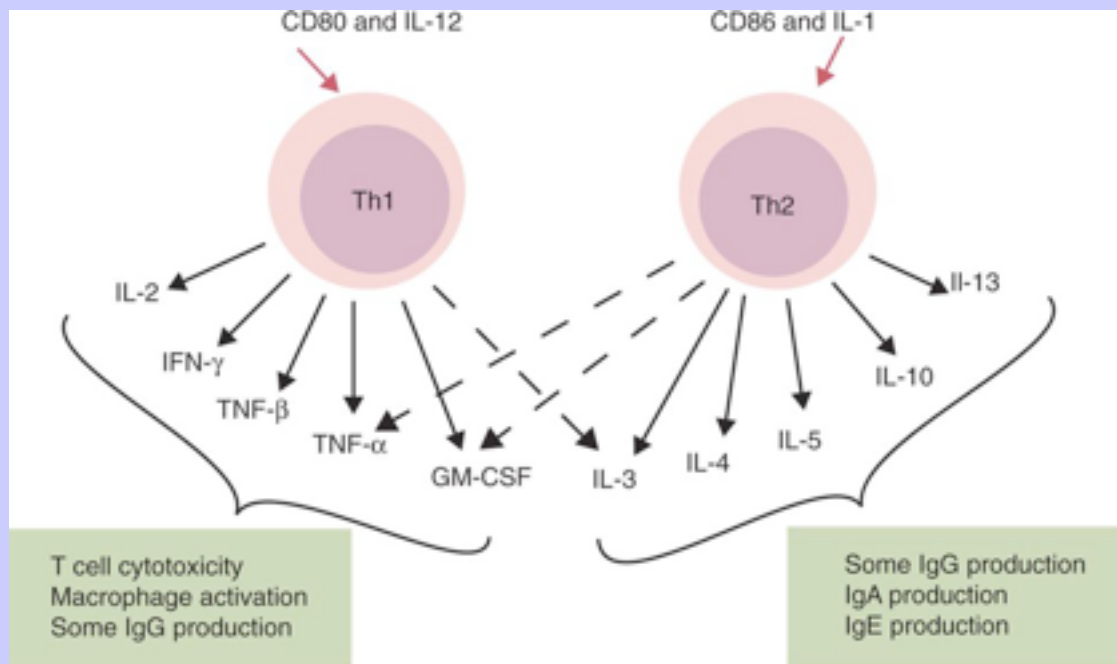


FIGURE 12-12 Major differences between Th1 and Th2. Note that the co-stimuli that trigger them are different, as are the set of cytokines they secrete.



co-stimulatory molecule CD80. The DC1 myeloid dendritic cells activate Th1 cells by secreting IL-12 and IL-18. Th1 cells then secrete IL-2, IFN-γ, TNF-α, and lymphotoxin (TNF-β) ([Figure 12-13](#)). Activated helper T cells secrete IL-2 and IFN-γ in a highly directed manner through the immunological synapse. They secrete TNF-α in all directions. Presumably the cytokines secreted through the synapse are for specific communication with other cells; those secreted multi-directionally promote inflammation and systemic responses. Th1 cells promote cell-mediated immune responses such as the delayed hypersensitivity reaction and macrophage activation. They thus generate immunity to intracellular organisms such as the mycobacteria and to viruses. In the absence of IL-12, the helper T cell response switches automatically from Th1 to Th2 ([Figure 12-14](#)).

12.9.2 Th2 Cells

Th2 cells respond optimally to antigen presented by plasmacytoid dendritic cells (DC2) (see [Chapter 8, Figure 8-7](#)) and macrophages, and less well to antigen presented by B cells. DC2 cells secrete IL-4 and provide co-stimulation through CD86. Th2 cells also have IL-1 receptors and may also require co-stimulation by IL-1 from macrophages or dendritic cells. Once activated, Th2 cells secrete IL-4, IL-5, IL-10, and IL-13 ([Figure 12-15](#)). The cytokines from Th2 cells stimulate B cell proliferation and immunoglobulin secretion but have no effect on delayed hypersensitivity or other cell-mediated reactions. The cytokines from Th2 cells enhance B cell production of IgG and IgA up to twentyfold and production of IgE up to 1000-fold. Th2 responses are associated with enhanced immunity to some parasitic worms such as *Toxocara canis* but with decreased resistance to mycobacteria and other intracellular organisms.

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Th1 and Th2 cells may migrate preferentially into different types of inflamed tissue as a result of differential expression of receptors for P- and E-selectin and the chemokine CCL11 (eotaxin). This may be important in ensuring that the correct T cell subpopulation is employed against a specific invader.

FIGURE 12-13 The cytokines produced by Th1 cells and their major properties.

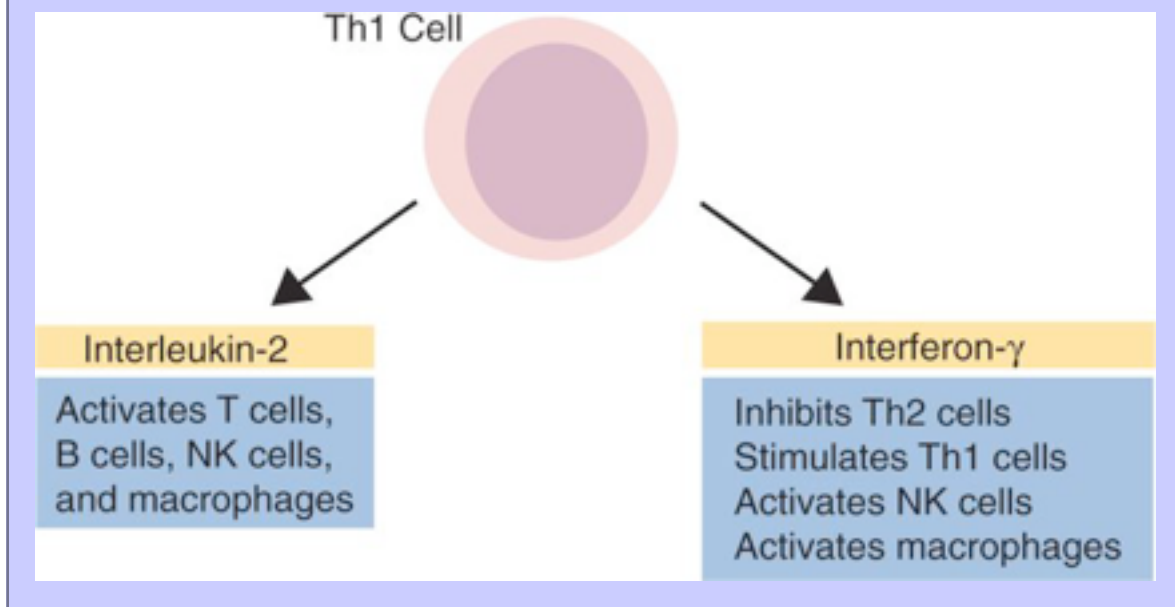


FIGURE 12-14 Different antigens can trigger distinctly different Th cell subpopulations. For example, T cells exposed to a parasite antigen from the roundworm *Toxocara canis* mount a Th2 response and secrete primarily interleukin-4 (IL-4) and IL-5. In contrast, T cells exposed to purified protein derivative (PPD), an antigen from *Mycobacterium tuberculosis*, mount a Th1 response characterized by secretion of interferon- γ (IFN- γ) and IL-2. (From Del Prete G, De Carli M, Mastromauro C, et al: *J Clin Invest* 88:346-350, 1991.)

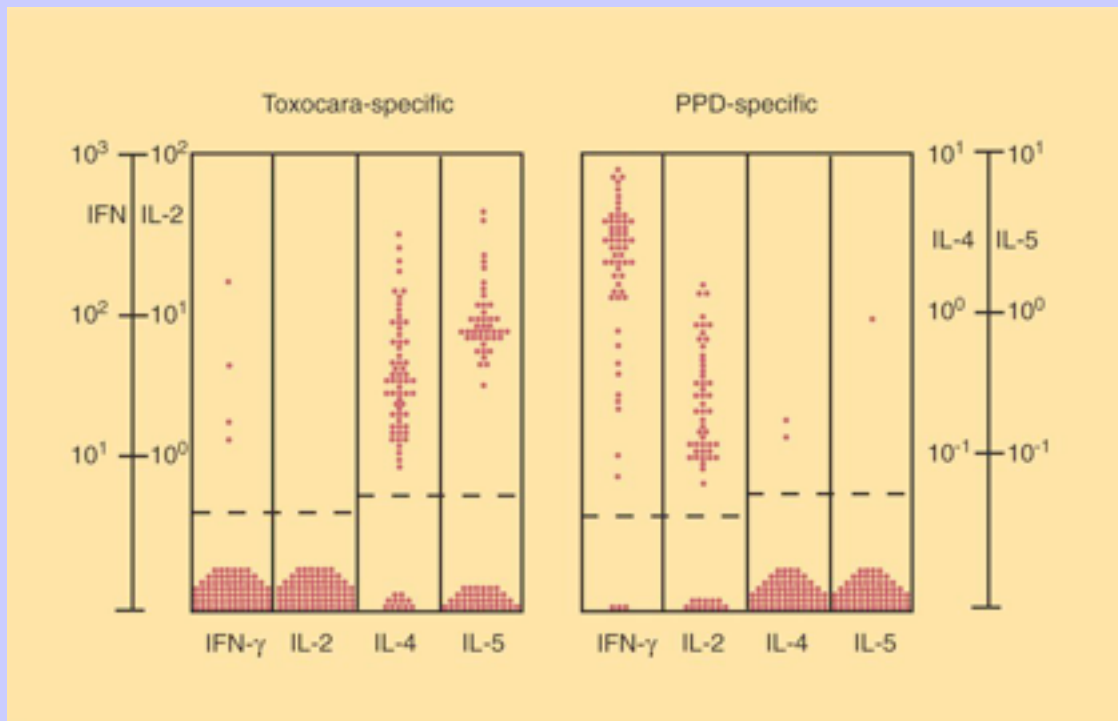
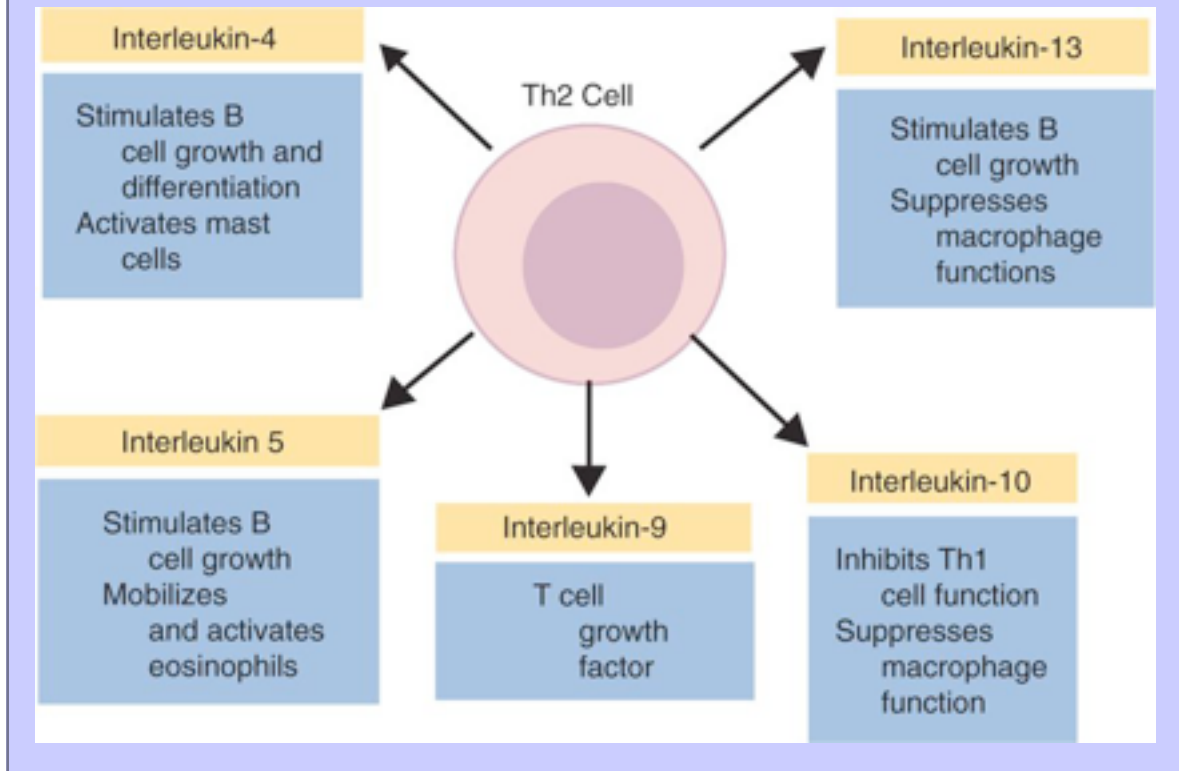


FIGURE 12-15 The cytokines produced by Th2 cells and their major properties.



12.9.3 Th0 Cells

Although the T cell subpopulations described above are usually considered to be discrete subsets, it has been argued that T cell cytokine profiles form a continuous spectrum with Th1 and Th2 cells as the extreme phenotypes. Thus some cells secrete a cytokine mixture that is a mixture of both Th1 and Th2. These cells, called Th0 cells, may be precursors of Th1 and Th2 or cells that are in transition between the two populations. Some IL-2-secreting T cells may switch to become IL-4-secreting cells after exposure to antigen, implying a change in phenotype from Th1 to Th2. The principal molecules that control this switch are IL-4 and IL-12. When cultured in the presence of IL-4, Th0 cells become Th2 cells. When cultured in the presence of IL-12, they become Th1 cells. Mixed (Th0) cell populations are most obvious early after initiation of an immune response, whereas Th1 and Th2 subsets are more obvious in chronic diseases where the antigens are persistent and cannot be easily removed.

12.9.4 Th17 Cells

A distinct population of CD4⁺ T cells that secrete IL-17 and promote some inflammatory reactions has recently been identified. The production of these cells is stimulated by the presence of dendritic cell-derived IL-6 and TGF- β together with either IL-23 or IL-21. Since Th17 cells themselves secrete IL-21, they promote their own development. Th17 cells are associated with inflammation in several autoimmune and chronic inflammatory

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diseases. Cytokines of the IL-17 family regulate immune responses adapted to clearing extracellular bacteria and fungi.

12.9.5 Species Differences

The details of helper T cell subpopulation function described above have largely been derived from studies in laboratory mice. Cattle most certainly possess Th1 and Th2 cells and can mount polarized immune responses. Bovine IgG1 expression is positively regulated by IL-4 and IgG2 expression by IFN- γ . A large proportion of bovine CD4⁺ cells produce multiple cytokines, including IL-2, IL-4, IL-10, and IFN- γ , and thus appear to be Th0 cells.

12.10 γ/δ T CELLS

The function of T cells bearing γ/δ TCRs remains an enigma as a result of major species differences. For example, only 5% to 15% of blood lymphocytes in humans and mice, but up to 60% of young ruminants, have γ/δ TCRs. It is probable that these cells have different functions in the two groups of mammals.

In humans and mice where γ/δ T cells are a minor lymphocyte subpopulation, they can be subdivided into subsets based on the diversity of their γ/δ antigen receptors. One subset has limited γ/δ receptor diversity and is mainly found in the skin and genital tract. The other subset has extensive receptor diversity and is mainly found in secondary lymphoid organs and intestinal mucosa. The skin T cells preferentially bind to common microbial PAMPs, especially heat-shock proteins and phospholipids (carbohydrates or nucleotides with a phosphate group). Other γ/δ T cells preferentially respond to the class Ib MHC molecules, MICA and MICB, both of which are produced by stressed cells, cancer cells, and virus-infected cells. When stimulated, some of these γ/δ T cells secrete fibroblast growth factor and so promote wound healing. The functions of the skin γ/δ cells may differ according to the stages of infection. Thus, early in infection, the cells with restricted antigen binding may serve an innate immune function and help resist intracellular bacteria such as *Mycobacterium* or *Listeria*. Later, in infections, they may serve an antiinflammatory or wound-healing role.

In contrast, the antigen-binding site on the other human γ/δ T cell subset binds to a diverse array of antigens, and these cells can recognize antigens directly without the need for an MHC molecule. These γ/δ T cells with diverse antigen-binding receptors form at least two populations. One population can be divided into Th1 and Th2 subsets, based on their secreted cytokines. The other population is cytotoxic and can destroy target cells, such as cells infected with mycobacteria and some leukemic cells. Since the vast majority of these cells are located on body surfaces, presumably they have a major defensive function. In humans, γ/δ T cells can also act as professional antigen-presenting cells. That is, they can capture and process antigens and present antigen fragments on MHC class II molecules to α/β T cells.

In young ruminants and pigs, γ/δ T cells are the major circulating lymphocyte population. They can bind a wide variety of antigens, suggesting that they have a role in acquired immunity. These cells colonize the skin, the mammary gland, the reproductive organs, and the intestinal wall, where they form the major T cell population. In pigs γ/δ T cells are polyclonal at birth, but their T cell diversity becomes increasingly restricted with age. In addition, γ/δ T cells located in different organs or even different parts of the gastrointestinal tract have different repertoires.

Ruminant γ/δ T cells are divided into WC1⁺ and WC1⁻ populations. The WC1⁺ cells are engaged in innate immunity, whereas the WC1⁻ cells are regulatory. The two subpopulations have a different tissue distribution. In mycobacterial and schistosomal infections, granulomas form around the invading organisms. In both cases the

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initial T cell infiltration is dominated by γ/δ T cells and is followed later by α/β T cells. A second wave of γ/δ T cells may terminate the response. These WC1+ γ/δ T cells secrete IL-12 and IFN- γ and may thus promote a Th1 bias in the immune response.

Human and bovine γ/δ T cells respond to microbial PAMPs by increasing chemokine gene expression. Thus lymphotactin (XCL1), macrophage inflammatory protein-1b, TNF- α , and granulocyte-macrophage colony-stimulating factor (GM-CSF) increase. The responding γ/δ T cells express TLR3, TLR9, mannose-binding lectin, and CD36. These γ/δ T cells may well be major contributors to innate immunity. Many γ/δ T cells have nonpolymorphic TCRs that recognize microbial glycolipids presented by CD1 positive antigen-presenting cells and release cytokines and lyse target cells just like conventional α/β T cells. The role of γ/δ T cells on mucosal surfaces is discussed further in [Chapter 19](#).

12.11 MEMORY T CELLS

When naïve T cells are stimulated by antigen under conditions that give rise to Th1 cells, two types of cell develop. One cell type secretes IFN- γ and serves as effector cells. These cells are short-lived because they are eliminated either by autocrine IFN- γ and IL-2 that trigger fas-mediated apoptosis or by nitric oxide produced by macrophages. Cells of the second type do not secrete IFN- γ ; they are resistant to apoptosis and develop into long-lived memory cells. This differentiation into two distinct populations is a result of asymmetrical T cell division. Thus T cells interact with antigen-presenting cells for several hours through an immunological synapse. Once it receives appropriate signals, the T cell undergoes mitosis and probably begins to divide before the two cells separate. The attached T cell is polarized: One pole of the cell contains the immunological synapse and associated structures; the other pole contains molecules excluded from the synapse. Thus when the T cell begins to divide, it forms two distinctly different daughter cells. The daughter cell adjacent to the synapse is the precursor of effector cells. The daughter cell formed at the distal pole, however, is the precursor of the memory cells. Memory T cells are themselves functionally heterogeneous. Thus central memory T cells remain in the secondary lymphoid tissues, such as lymph nodes awaiting the arrival of invaders, while other effector memory T cells are found in inflamed tissues, where they immediately attack invaders. Memory CD4⁺ and CD8⁺ T cells persist in the absence of antigen. These cells slowly divide and replenish their numbers. The cytokines IL-7 and IL-15 are required for the survival of memory CD8⁺ T cells, whereas only IL-7 is required for the survival of CD4⁺ T cells. In humans, memory CD4⁺ T cells have a half life of 8 to 12 years while memory CD8⁺ T cells have a half-life of 8 to 15 years. On the other hand, some individuals may lose their memory CD8⁺ T cells very rapidly for unknown reasons.

Human memory T cells express TLR2. If exposed to its ligand, lipopeptide in the presence of either IL-2 or IL-15, they will proliferate. Thus it is possible that bacterial PAMPs such as lipopeptide may promote the long-term survival of memory T cells even in the absence of persistent antigen.

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12.12 SOURCES OF ADDITIONAL INFORMATION

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13 CHAPTER 13 B Cells and Their Response to Antigen

13.1 KEY POINTS

- B cells possess antigen-binding receptors (B cell antigen receptors [BCRs]).
- When BCRs are shed into body fluids they are called immunoglobulins or antibodies.
- BCRs consist of two heavy and two light chains bound together by disulfide bonds.
- B cells can recognize most antigens without prior processing. However, an optimal B cell response normally requires stimulation by helper T cells.
- Helper T cells provide co-stimulation through a synapse involving co-stimulatory molecules and cytokines.
- Responding B cells may become either memory cells or antibody-secreting plasma cells.
- Plasma cells are the progeny of B cells modified to secrete very large amounts of antibodies.
- The differentiation of B cells into plasma cells takes place in the germinal centers of lymph nodes.
- Malignant plasma cells, called myeloma cells, produce large quantities of very pure immunoglobulin. If fused with a normal plasma cell, the resulting hybridomas can be made to produce large quantities of pure monoclonal antibodies.

The division of the acquired immune system into two major components is based on the need to recognize two distinctly different forms of foreign invaders. Some invaders enter the body openly and grow in extracellular fluids. These exogenous antigens are destroyed by antibodies. Other invaders grow inside cells, where antibodies cannot reach. They are destroyed by T cell-mediated responses. Antibodies are produced by the lymphocytes, called B cells. This chapter discusses B cells and their response to antigens.

B cells are found in the cortex of lymph nodes, in the marginal zone in the spleen, in the bone marrow, throughout the intestine, and in Peyer's patches. Few B cells circulate in the blood. Like T cells, each B cell has a large number of identical antigen-binding receptors on its surface. Each B cell therefore can only bind and respond to a single antigen. Antigen receptors are generated at random during B cell development in a process described in [Chapter 15](#). If a B cell encounters an antigen that can bind to its receptors, it will, with appropriate co-stimulation, respond by secreting receptor molecules into body fluids, where they are called antibodies. Each B cell thus makes antibodies of the same binding specificity as its receptors. This specificity is the result of a series of random gene rearrangements, all of which must be successfully performed if the B cell is to survive. In addition, during an immune response a second selection process occurs in which B cell receptors are modified by random somatic mutation or gene conversion. Only B cells with receptors that can bind an antigen with a high affinity will survive to become memory cells.

13.2 THE B CELL ANTIGEN RECEPTOR

Each B cell is covered with about 200,000 to 500,000 identical antigen receptors (B cell antigen receptors [BCRs]), many more than the 30,000 T cell antigen receptors (TCRs) expressed on each T cell. Each BCR is constructed from multiple peptide chains and, like the TCR, can be divided into antigen-binding and signaling components. Unlike the TCR, however, the BCR can also bind antigens when released from the B cell surface. Antibodies are

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simply soluble BCRs secreted into body fluids; they all belong to the family of proteins called immunoglobulins (see [Chapter 12](#)).

13.2.1 The Antigen-Binding Component

The antigen-binding component of the BCR (or immunoglobulin) is a glycoprotein of 160 to 180 kDa, consisting of four linked peptide chains. These four chains consist of two identical pairs—a pair of heavy chains, each 60 kDa in size, and a pair of smaller chains, about 25 kDa each, called light chains ([Figure 13-1](#)). The light chains are linked by disulfide bonds to the heavy chains so that the complete molecule is shaped like the letter Y. The tail of the Y (called the Fc region) is formed from paired heavy chains and is attached to the lipid bilayer of the B cell surface. The arms of the Y (called the Fab regions) are formed by paired light and heavy chains, and they bind antigens ([Figure 13-2](#)). The antigen-binding sites are formed by the grooves between light and heavy chains. Thus each BCR has two identical antigen-binding sites.

13.2.1.1 Light Chains

Light chains are constructed from two domains, each containing about 110 amino acids. The amino-acid sequences in the C-terminal domain of light chains from different B cells are identical and so form a constant domain (C_L). In contrast, the sequences in the N-terminal domain differ in each cell examined and so form a variable domain (V_L).

Mammals make two distinct types of light chains, called κ (kappa) and λ (lambda). Although their amino acid sequences are different, they are functionally identical. The ratio of κ to λ chains in BCRs varies among mammals, ranging from mice and rats, which have more than 95% κ chains, to cattle and horses, which have 95% λ chains. Primates such as the rhesus monkey or the baboon have 50% of each, whereas humans have 70% κ chains. Carnivores such as cats and dogs have 90% λ chains.

13.2.1.2 Heavy Chains

The heavy chains of a typical immunoglobulin contain 400 to 500 amino acids. They consist of four or five domains, each of which consist of about 110 amino acids. The N-terminal domain has a highly variable sequence and is therefore called the variable (V_H) domain. The remaining three or four domains show few differences and so form constant (C_H) domains.

FIGURE 13-1 The overall structure of an immunoglobulin molecule. When bound to a B cell surface, this molecule acts as an antigen receptor (B cell antigen receptor). When released by the B cell and free in the circulation, it acts as an antibody. Note that unlike a T cell antigen receptor, it has two antigen-binding sites.

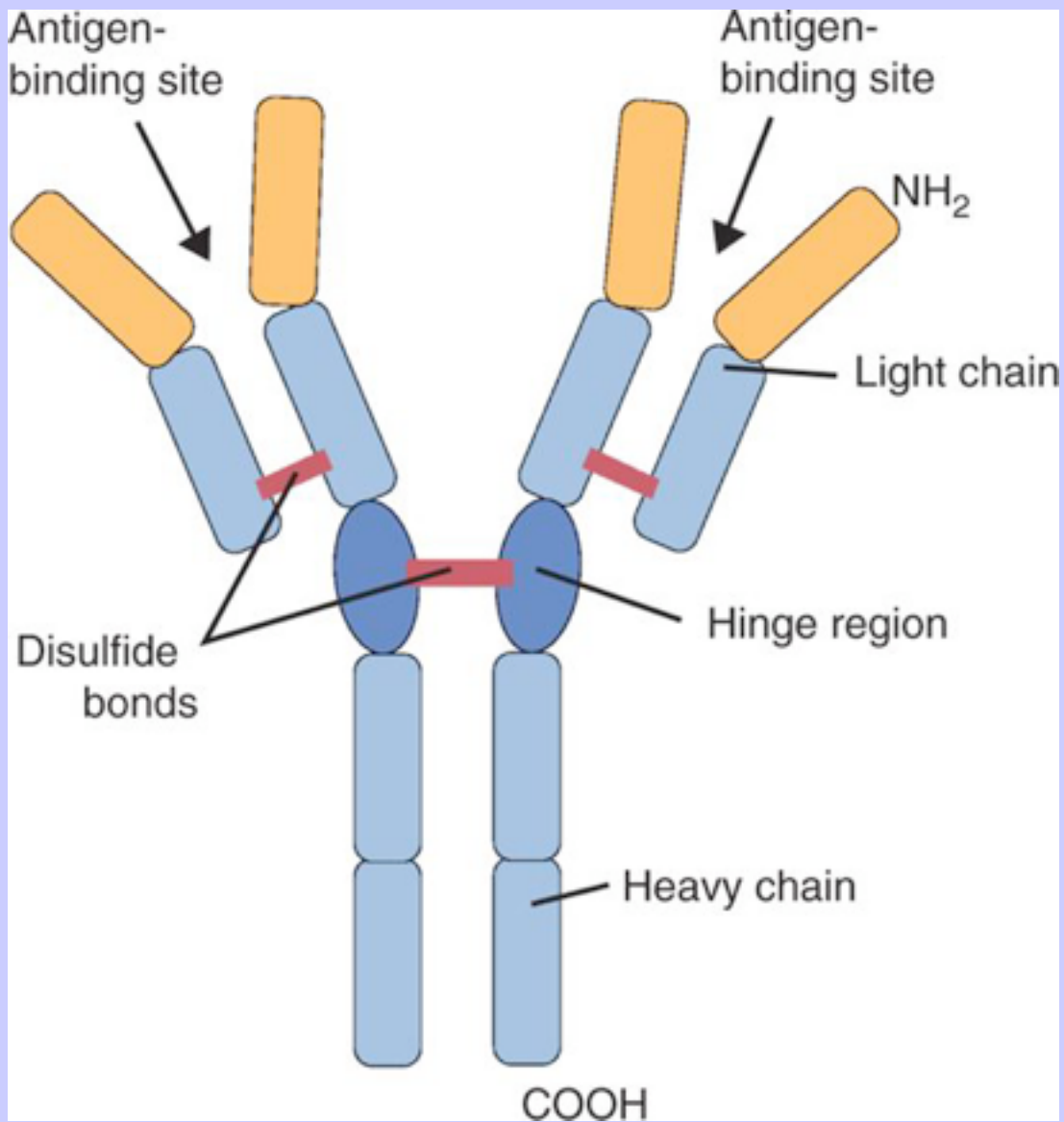
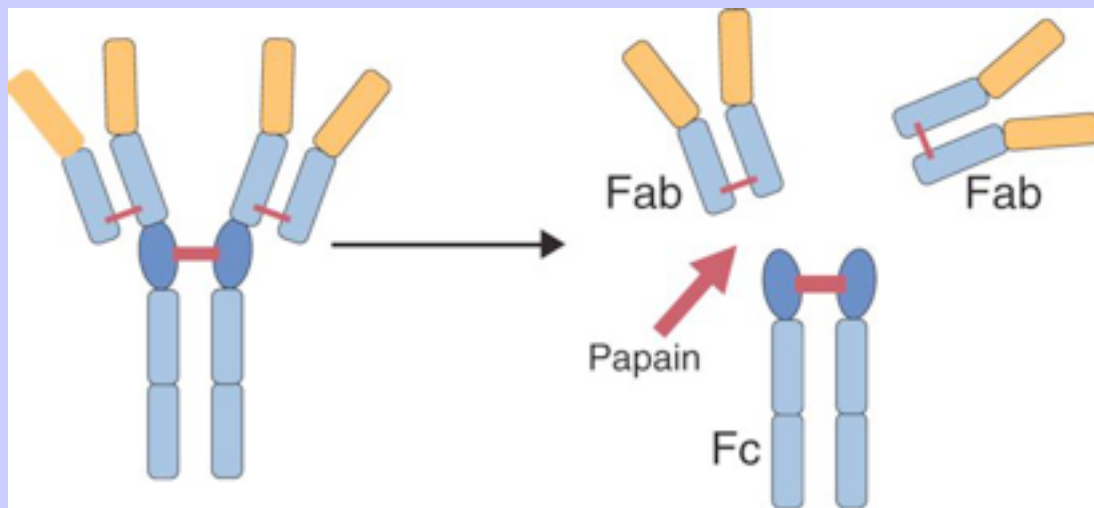


FIGURE 13-2 The effect of treating an immunoglobulin molecule with the proteolytic enzymes, pepsin, or papain. Papain cleaves the molecule into three large fragments. Pepsin cleaves the molecule into one large fragment and many small ones. The names of these fragments denote the nomenclature of different regions of an immunoglobulin molecule.



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B cells make five different classes of heavy chain that differ in their amino acid sequence and in their domain structure. As a result, each immunoglobulin class has a different biological activity. The five distinct immunoglobulin heavy chains are called α , γ , δ , ϵ , and μ . These heavy chains determine the immunoglobulin class (or isotype). Thus immunoglobulin molecules that use α heavy chains are called immunoglobulin A (IgA); those that use γ chains are called IgG; μ chains are used in IgM, δ chains in IgD, and ϵ chains in IgE.

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13.2.1.3

Variable Regions

When the amino-acid sequences of a large number of V domains from light and heavy chains are examined in detail, two features become apparent. First, their sequence variation is largely confined to three small regions, each consisting of 6 to 10 amino acids, within the variable domain ([Figure 13-3](#)). These regions are said to be hypervariable. Between the three hypervariable regions are relatively constant sequences of amino acids called framework regions. The three hypervariable regions on each chain determine the shape of the antigen-binding site and so determine the specificity of antigen binding. Since the shape of the antibody-binding site is complementary to the conformation of the antigenic determinant, the hypervariable sequences are also called complementarity-determining regions (CDRs). Each V-domain is folded in such a way that its three CDRs come into close contact with the antigen ([Figure 13-4](#)).

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13.2.1.4

Constant Regions

The number of constant domains differs among immunoglobulin classes. For example, there are three constant domains in a γ heavy chain: they are labeled, from the N-terminal end, as C_H1 , C_H2 , and C_H3 . A similar arrangement is found in α and most δ chains, whereas μ and ϵ chains have an additional constant domain called C_H4 .

Since heavy chains are paired, the domains in each chain come together to form structures by which antibody molecules can exert their biological functions. Thus V_H and V_L form a paired domain that binds antigen, and C_H1 and C_L together stabilize the antigen-binding site. The paired C_H2 domains of IgG contain a site that activates the classical pathway of the complement system (see [Chapter 5](#)) and a site that binds to Fc receptors on phagocytic cells ([Figure 13-5](#)). The heavy chain also regulates the transfer of IgG across the placenta and antibody-mediated cellular cytotoxicity (see [Chapter 16](#)). When immunoglobulin molecules serve as BCRs, part of their Fc region is embedded in the B cell surface membrane. These cell-bound immunoglobulins differ from the secreted form in that

FIGURE 13-3 The variable regions of the light and heavy chains of an immunoglobulin molecule are divided into three highly variable complementarity-determining regions separated by relatively constant framework regions.

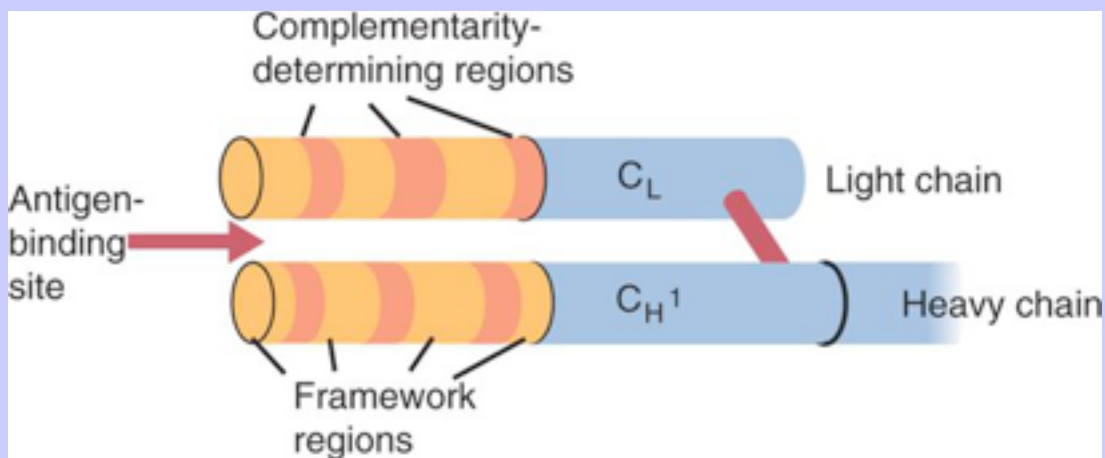


FIGURE 13-4 The way in which the complementarity-determining regions are folded to form the antigen-binding site on an immunoglobulin molecule. A similar folding occurs in the peptide chains of the T cell antigen receptor.

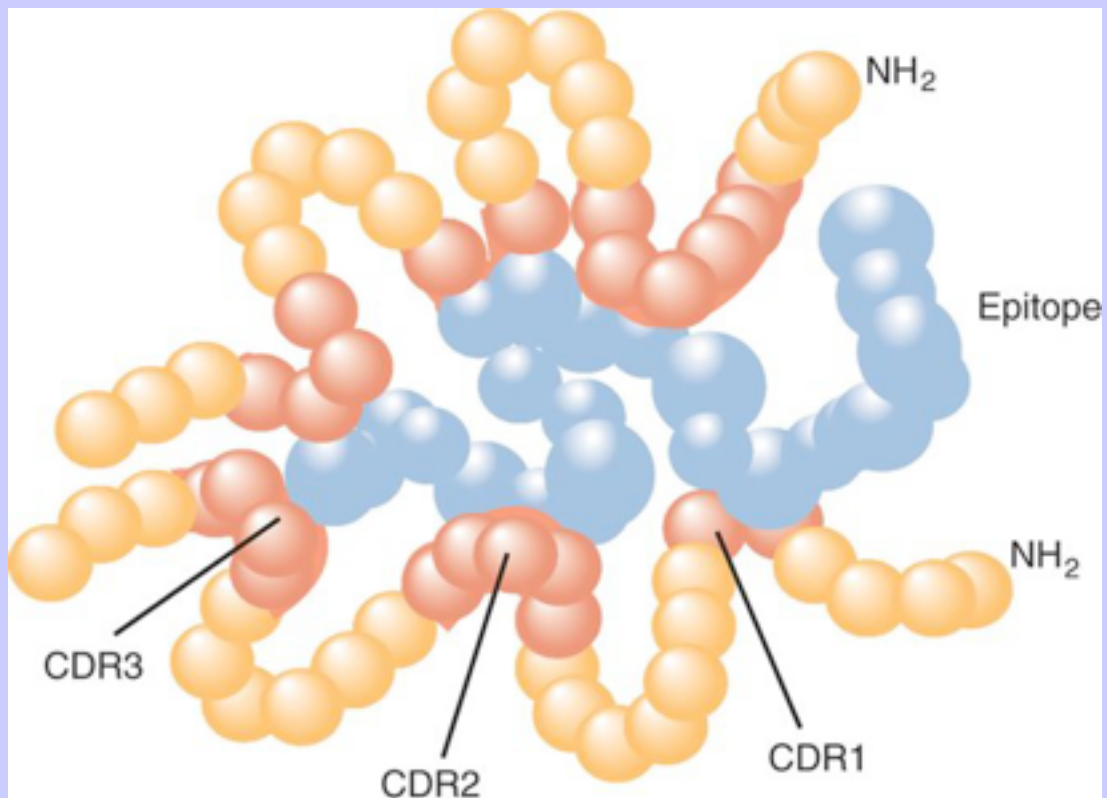
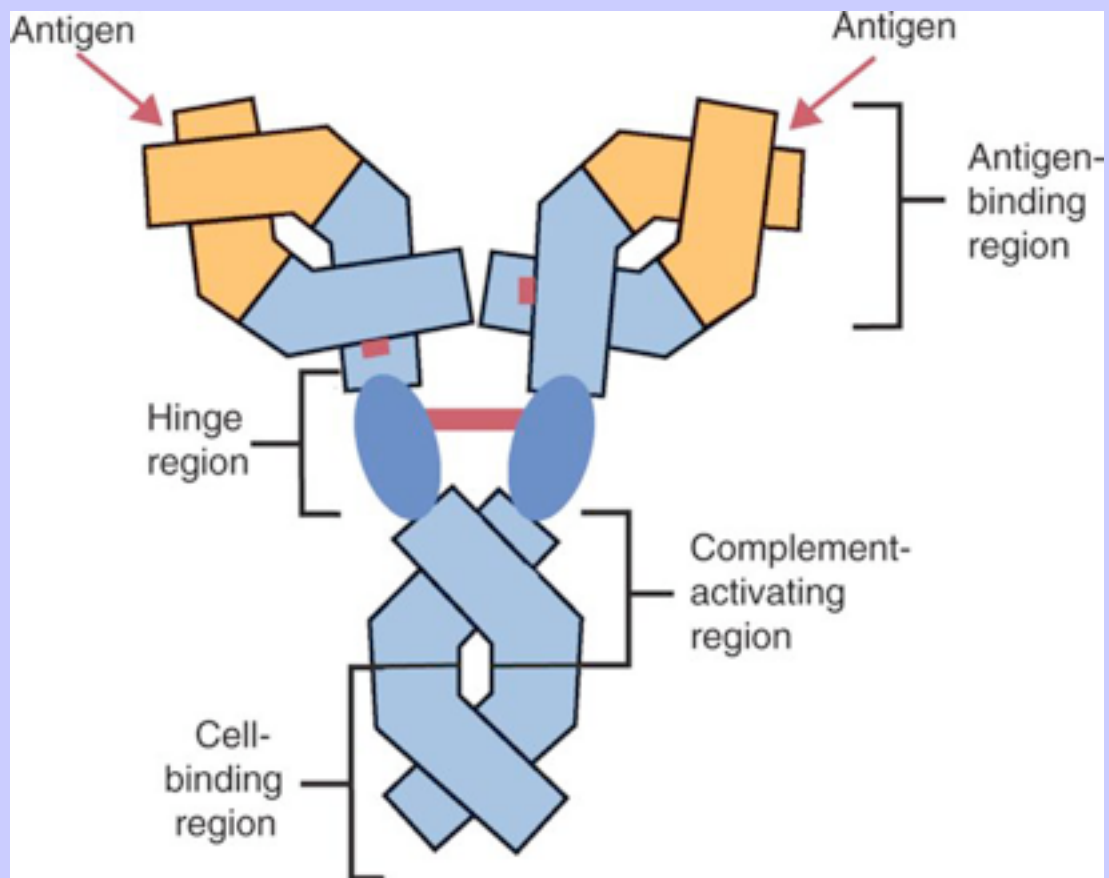
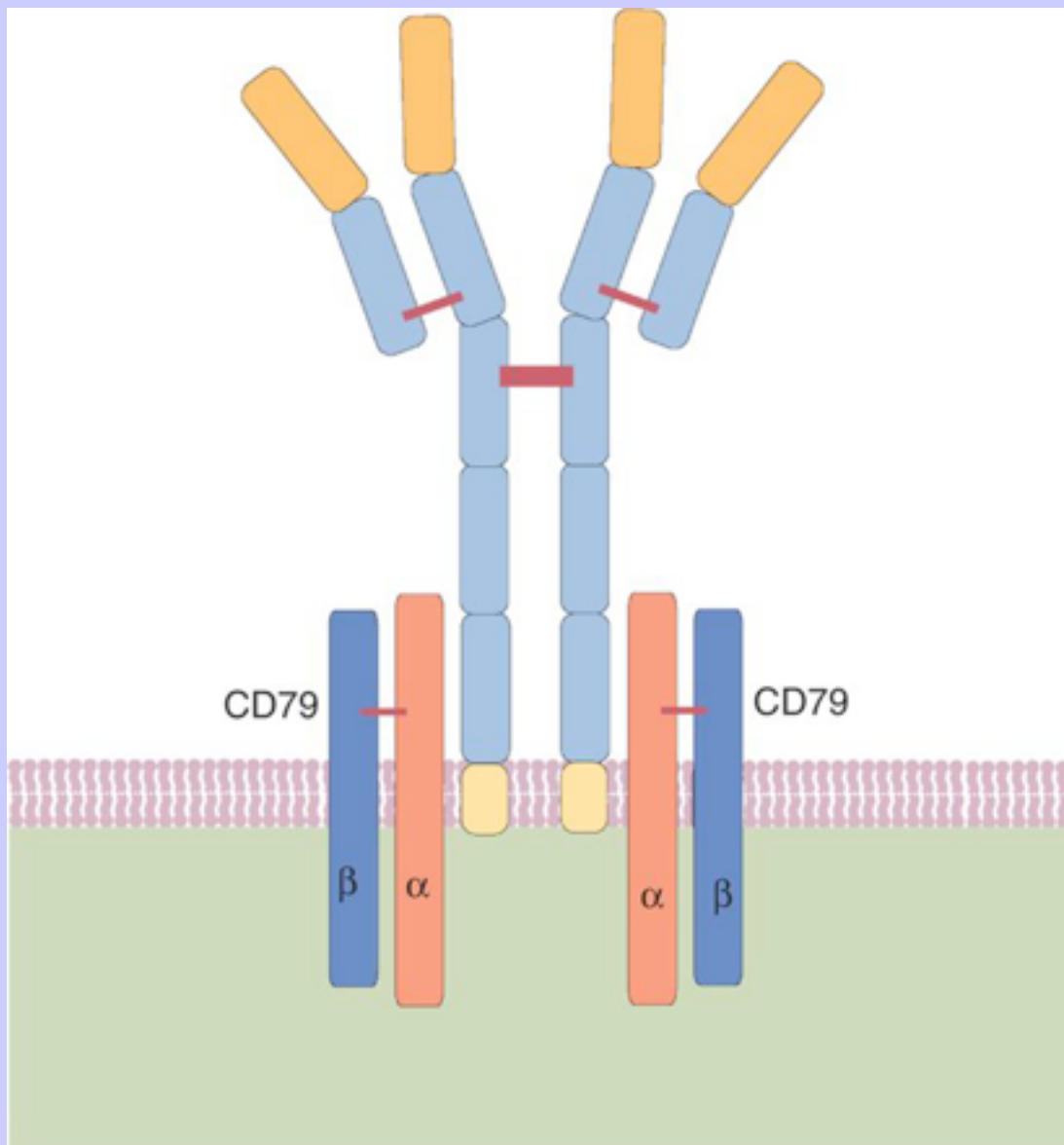


FIGURE 13-5 The structure of an immunoglobulin G molecule, showing how the light and heavy chains intertwine to form clearly defined regions of the molecule. Each region has defined biological functions.



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FIGURE 13-6 The structure of a complete B cell antigen receptor, showing both the antigen-binding component (immunoglobulin) and the signal-transducing components (CD79). Note the small transmembrane domain at the end of each heavy chain.



they have a small transmembrane domain located at their C-terminus. This contains hydrophobic amino acids that associate with the cell-membrane lipids.

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13.2.1.5 Hinge Region

One important feature of the immunoglobulins is that the Fab regions, which contain the antigen-binding sites, can swing freely around the center of the molecule as if they are hinged. This hinge consists of a small domain of about 12 amino acids located between the C_H1 and C_H2 domains. The hinge region contains many hydrophilic and proline residues that cause the peptide chain to unfold and make this region readily accessible to proteases. This region also contains all the interchain disulfide bonds. Proline, because of its configuration, produces a 90-degree bend when inserted in a polypeptide chain. Because amino acids can rotate around peptide bonds, the effect of closely spaced proline residues is to produce a universal joint around which the immunoglobulin chains can swing freely. The μ chains of IgM do not possess a hinge region.

13.2.2 The Signal-Transducing Component

B cell receptor immunoglobulins cannot signal directly to their B cell since their cytoplasmic domains contain only three amino acids. However, their C_H4 and transmembrane domains associate with glycoprotein heterodimers formed by pairing CD79a (Ig- α) (47 kDa) and CD79b (Ig- β) (37 kDa). These act as signal transducers ([Figure 13-6](#)). The CD79b chains are identical in all BCRs. The CD79a chains differ depending on their associated heavy chains and so employ different signaling pathways.

BCR signaling is initiated by antigen binding. This leads to receptor aggregation and subsequent phosphorylation of the immunoreceptor tyrosine-based activation motifs (ITAMs) on the cytoplasmic domains of CD79a and CD79b. Phosphorylation of the tyrosines in these motifs by the Src family kinases lyn, fyn, or blk results in recruitment of another kinase called syk. Syk initiates several downstream signaling cascades (see [Chapter 6](#)).

13.3 CO-STIMULATION OF B CELLS

B cells require multiple signals if they are to be fully activated ([Figure 13-7](#)). Thus although the binding of antigen to a BCR is an essential first step in triggering a B cell response, it is usually insufficient to trigger antibody formation. Complete activation of a B cell requires co-stimulation by the helper T cells. In order to do this, however, the helper T cells must themselves be presented with antigen. This antigen can be presented by one of the professional antigen-presenting cells, a dendritic cell, a macrophage, or even a B cell. Thus a B cell can capture and process antigen, present it to a T cell, and then receive co-stimulation from the same T cell. B cells thus play two simultaneous roles. They respond to antigen by making antibodies while at the same time acting as antigen-processing cells. The helper T cells provide the B cell with co-stimulatory signals from cytokines, as well as from interacting receptor pairs.

13.3.1 Antigen Presentation by B Cells

B cells are effective antigen-presenting cells. Following antigen binding, the BCR may be internalized and degraded or transported to an intracellular compartment, where newly synthesized major histocompatibility complex (MHC) class II molecules and antigen fragments are bound together to form complexes. These peptide-MHC class II complexes are carried to the B cell surface, where they form a synapse with helper T cells (see [Figure 13-8](#)). This activates the T cells, which then provide co-stimulation to the B cell and permit its full activation.

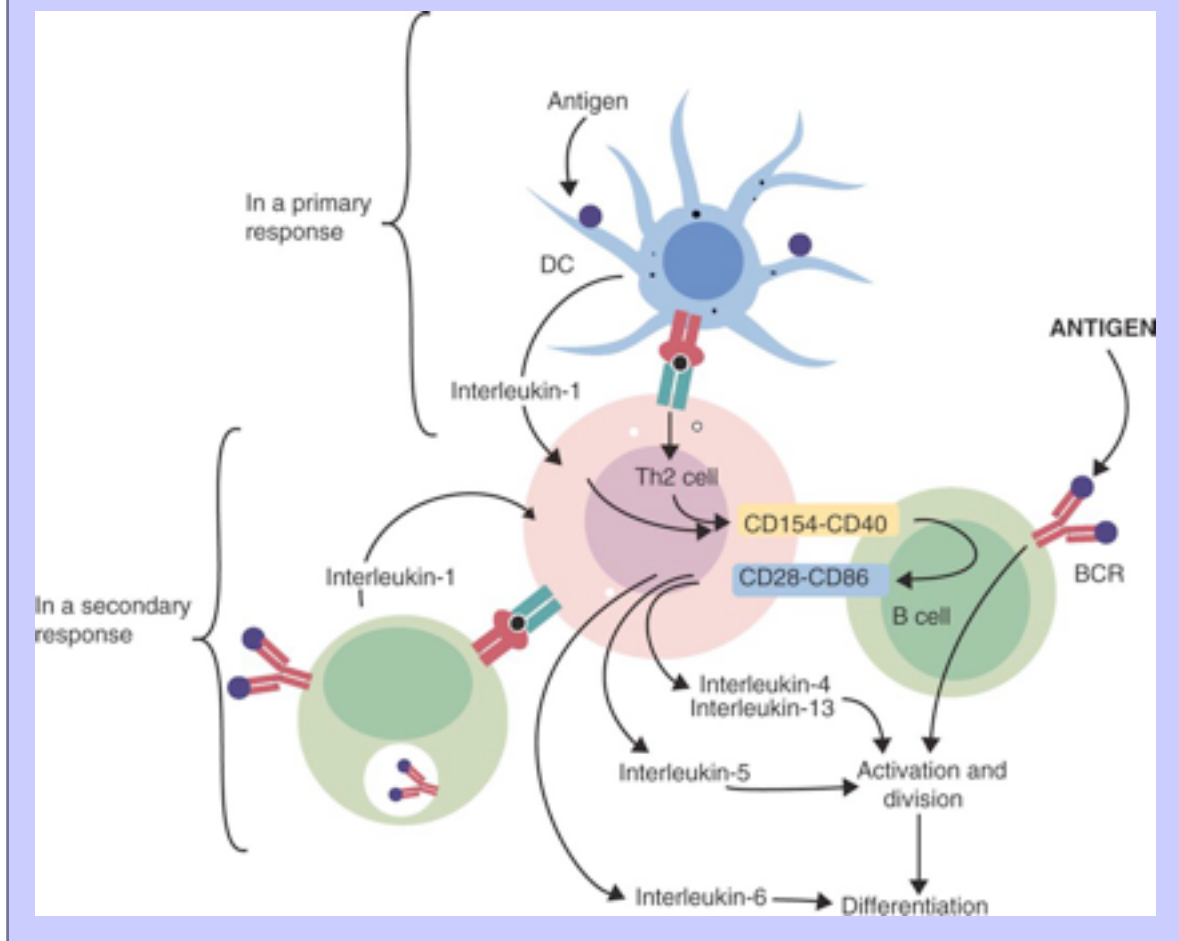
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Since all the receptors on a single B cell are identical, each B cell can bind only one antigen. This makes them much more efficient antigen-presenting cells than macrophages, which must ingest any foreign material that comes their way. This is especially true

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FIGURE 13-7 The sequence of events that must occur for a B cell to respond to antigen. Not only must the B cell be stimulated by antigen, but it must also receive co-stimulation from helper T cells and their cytokines. This complex interaction can be seen in [Chapter 8, Figure 8-6, D.](#)



in a primed animal, in which large numbers of B cells can bind a specific antigen. B cells can thus activate Th cells with 0.1% of the antigen concentration of nonspecific antigen-processing cells such as macrophages.

Table 13-1 The Immunoglobulins Produced by B Cells in the Presence of Th1 and Th2 Antigen-Specific Helper T Cell Clones in Mice

Class	Th1 Cells (ng/ml)	Th2 Cells (ng/ml)
IgG1	<8	21,600
IgG2a	14	39
IgG2b	<8	189
IgG3	<8	354
IgM	248	98,000
IgA	<1	484
IgE	<1	187
Adapted from Coffmann RL et al: <i>Immunol Rev</i> 102:5, 1988.		

13.3.2 Cytokine Secretion

Th2 cells secrete cytokines that initiate B cell activation and differentiation. The four most important are interleukin-4 (IL-4), IL-5, IL-6, and IL-13.

IL-4 is produced by activated Th2 cells, mast cells, and activated basophils. It stimulates the growth and differentiation of B cells. It also enhances their expression of MHC class II and Fc receptors. IL-4 also induces B cells to switch immunoglobulin class synthesis. For example, it stimulates IgE production (Table 13-1). The actions of IL-4 are neutralized by interferon- γ (IFN- γ). As a result, IFN- γ inhibits IgE synthesis and B cell proliferation.

IL-5 acts on activated B cells to promote their differentiation into plasma cells. It stimulates IgG and IgM production and enhances IL-4-induced IgE production. IL-5 selectively stimulates IgA production in mucosal B cells.

IL-13 has biological activities similar to those of IL-4 because it acts through a receptor (CD213) that shares a common a chain with the IL-4R. It has similar effects to IL-4 on B cells, stimulating their proliferation and increasing immunoglobulin secretion. IL-13 is required for optimal induction of IgE, especially if IL-4 is low or absent.

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IL-6 is needed for the final differentiation of activated B cells into plasma cells. It acts together with IL-5 to promote IgA production and with IL-1 to promote IgM production.

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13.3.3 CD40 and CD154

Cytokines alone cannot fully activate B cells. Successful co-stimulation also requires cell-cell interactions mediated through a synapse by receptor pairs such as CD40 and CD154. The co-stimulatory molecule CD40 is expressed on resting B cells, whereas its ligand, CD154, is expressed on activated helper T cells. The interaction of CD40 with CD154 is required for a B cell to begin its cell cycle and upregulate the expression of IL-4 and IL-5 receptors (Figures 13-8 and 13-9). The signals from CD40 synergize with IL-4 and IL-5 to drive B cell

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In order to fully respond to antigen, B cells must also receive signals transmitted through a CD21-CD19 complex on the B cell surface. CD21 is a complement receptor (CR2) that can bind to its ligand, C3d. CD19 is its accompanying signaling component. If an antigen molecule has C3d attached to it, this binds to the CD21 and a signal is transmitted via CD19 to the BCR ([Figure 13-10](#)). The signals generated by each receptor synergize so that stimulation of both a BCR and CD19/CD21 lowers the threshold for B cell activation 100-fold. The importance of complement in stimulating B cells is

FIGURE 13-8 The sequence of events that occurs when an antigen-processing B cell interacts with a helper T cell. During a primary immune response, antigen is processed by a dendritic cell and presented to the helper T cell. During a secondary immune response, the B cell itself can act as an antigen-presenting cell. Co-stimulators, such as CD154 and CD28, engage serially to trigger interleukin-4 (*IL-4*) secretion by the T cell and *IL-4R* production by the B cell.

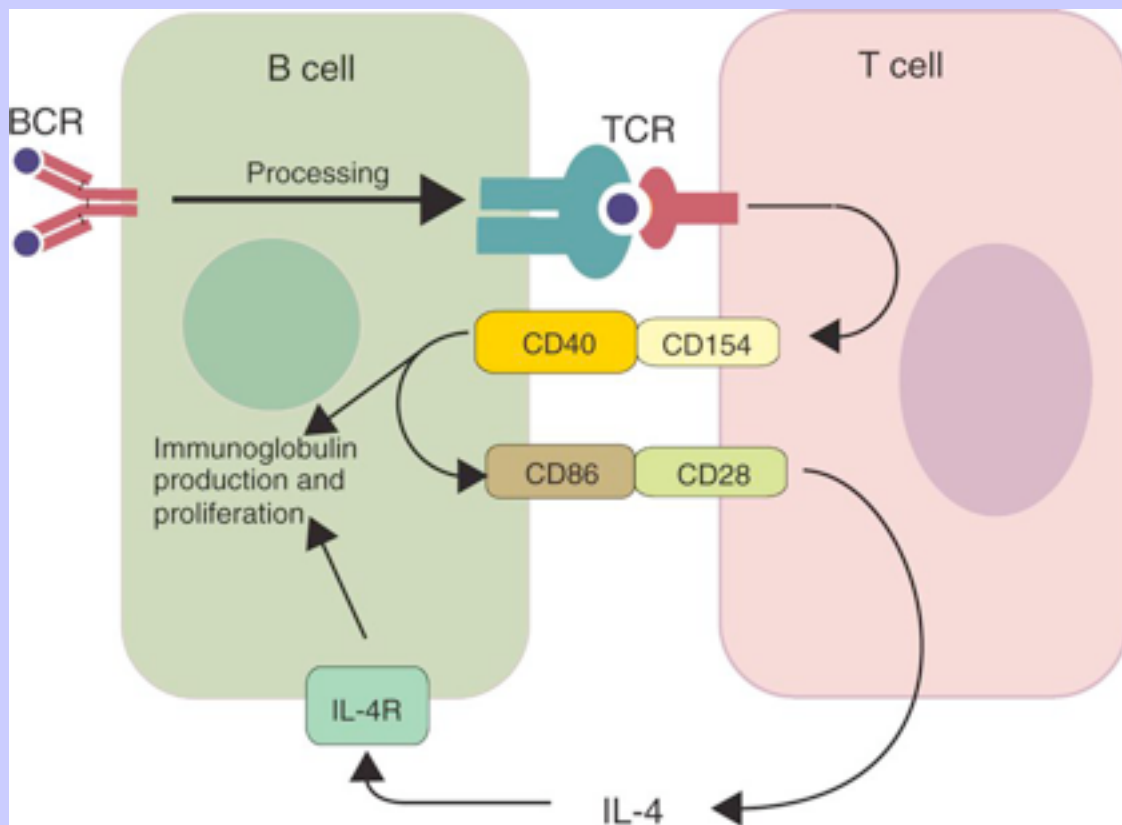
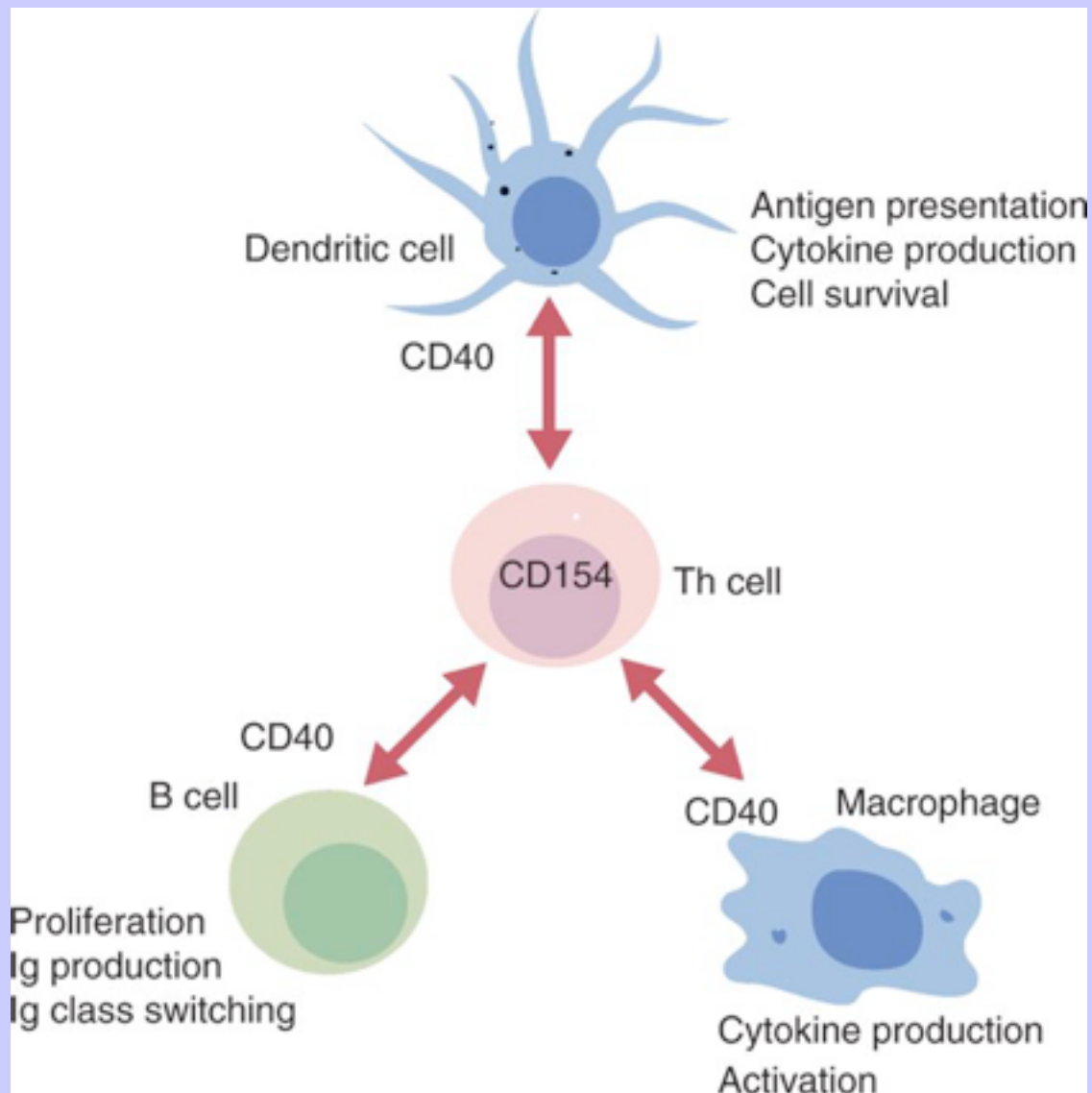
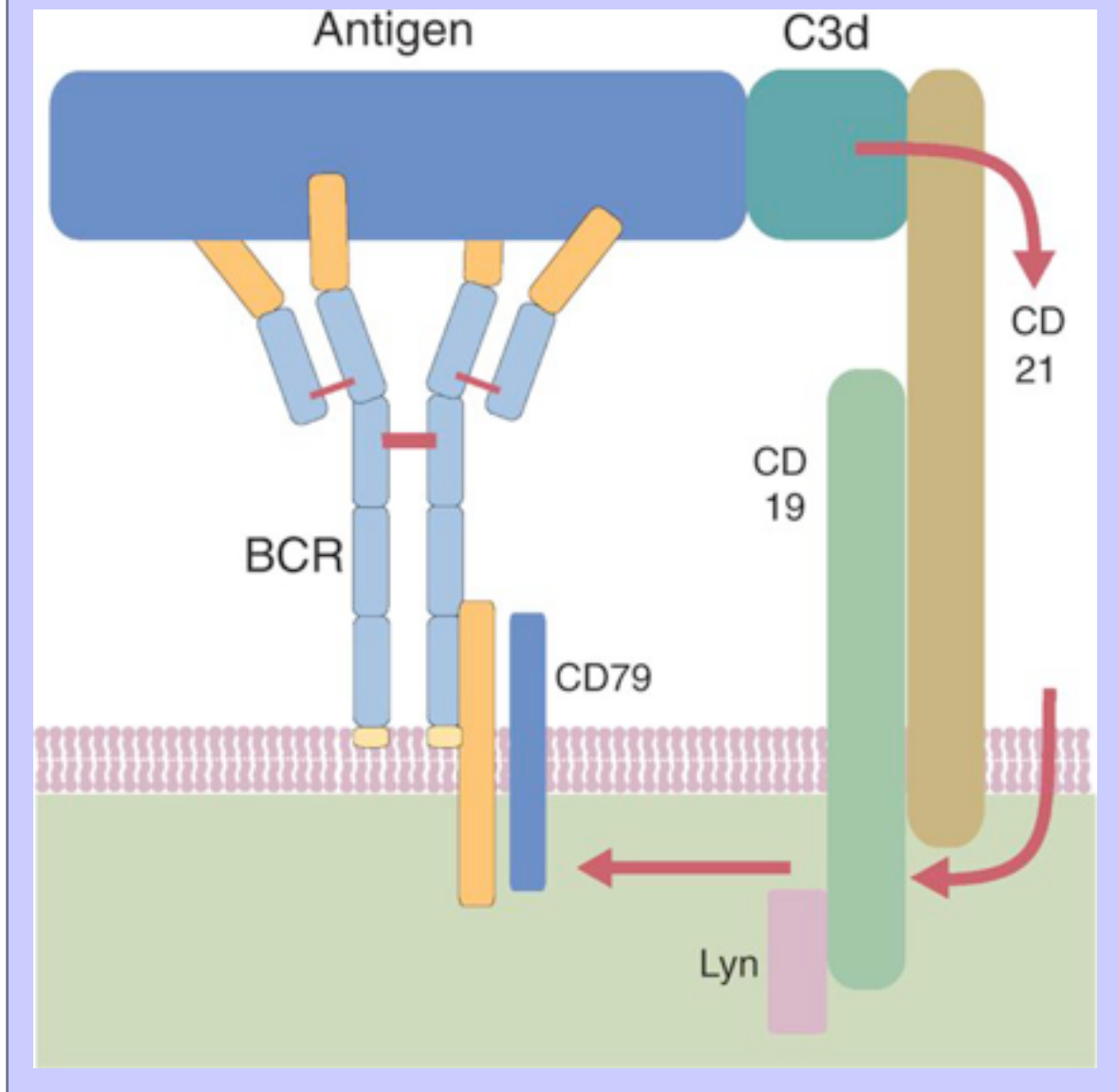


FIGURE 13-9 CD40 and CD154 participate in a dialog between T cells and the professional antigen-presenting cells. As a result, both cell types are stimulated. In the case of B cells, T cell stimulation permits B cell proliferation and immunoglobulin production.



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FIGURE 13-10 The stimulation of B cells through the CD21/CD19 complex. CD21 binds to C3d on the antigen. Signaling through CD19, it generates a potent co-stimulatory signal to enhance B cell responses.



emphasized by the observation that mice deficient in some complement components (C3, C4, or CR2) cannot mount an effective antibody response to T-dependent antigens.

The B cell Fc receptor, FcγRIIb, is a negative regulator of B cell function. When an IgG molecule binds to it and cross-links to a BCR through antigen, it inhibits antibody formation. This has important practical consequences when vaccinating young animals (see [Chapter 18](#)).

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13.3.5 TLRs and PAMPs

Although BCR stimulation and T cells help trigger initial B cell division, they cannot induce a prolonged, self-sustaining B cell response. This requires toll-like receptor (TLR) stimulation.

TLRs play a key role in stimulating acquired immune responses. For example, germfree newborn piglets cannot respond effectively to antigens unless they are first colonized by bacteria. Signaling through TLR4 promotes precursor B cell maturation while signaling through TLR2 seems to retard B cell development. Analysis shows that pathogen-associated molecular patterns (PAMPs) such as muramyl dipeptide, lipopolysaccharides, and CpG oligonucleotides either individually or collectively can induce antibody responses and that some combinations can induce class switching.

In addition to promoting the development of the neonatal B cell system, TLRs also promote B cell responses to antigens when exposed to microbial PAMPs. In fact, complete activation of B cells requires the activation of their TLRs. The B cell-stimulating ligands include flagellins, lipopolysaccharides, and CpG DNA. Activation of TLR4 enhances B cell antigen presentation, promotes germinal center formation, and is required for optimal antibody production against T dependent antigens (at least for some IgG subclasses). This response is inhibited by the presence of TLR2. TLR signaling to memory B cells increases antibody production. TLR stimulation does not appear to be required for IgA and IgE production. Thus TLR signaling can substitute in part for T cell help, which explains why antibody production still occurs in AIDS patients despite their lack of T cells. The adaptor molecule MyD88 is required for TLR signaling, since in MyD88 knockout mice the production of IgM, IgG1, and IgG2c is significantly impaired. This knockout has no apparent direct effect on IgG3, IgA, or IgE production. TLR4 knockout mice also show impaired IgM responses. This further demonstrates the overlap between the innate and acquired immune systems.

13.4 THE B CELL RESPONSE

Binding of antigen to the BCR, especially if two receptors are cross-linked, exposes ITAMs, triggers activation of several different tyrosine kinases, and results in phosphorylation of a phospholipase C and possibly a G-protein ([Figure 13-11](#)). As with the TCR, these reactions are dynamically regulated by CD45 tyrosine phosphatases. Subsequent hydrolysis of phosphatidylinositol and calcium mobilization leads to activation of a protein kinase C and calcineurin and activation of the transcription factors fos and myc.

13.4.1 Differential Signaling

Like the TCR, the BCR probably produces a tunable signal. As a result, it triggers biological responses that differ depending on the properties of the antigen and the amount of co-stimulation received. Receptor affinity can influence B cell proliferation and antibody secretion. On the other hand, receptor occupancy influences MHC class II expression and signal transduction. The precise direction of the immunoglobulin class switch will depend on whether the B cell is exposed to Th1 or Th2 cytokines.

Certain antigens can provoke antibody formation in the absence of helper T cells. These so-called T-independent antigens are usually simple repeating polymers (and PAMPs) such as *Escherichia coli* lipopolysaccharide, polymerized salmonella flagellin, and pneumococcal polysaccharide. These T independent

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FIGURE 13-11 Signal transduction by two cross-linked B cell antigen receptors activates B cells triggering cell division, differentiation, and immunoglobulin synthesis.

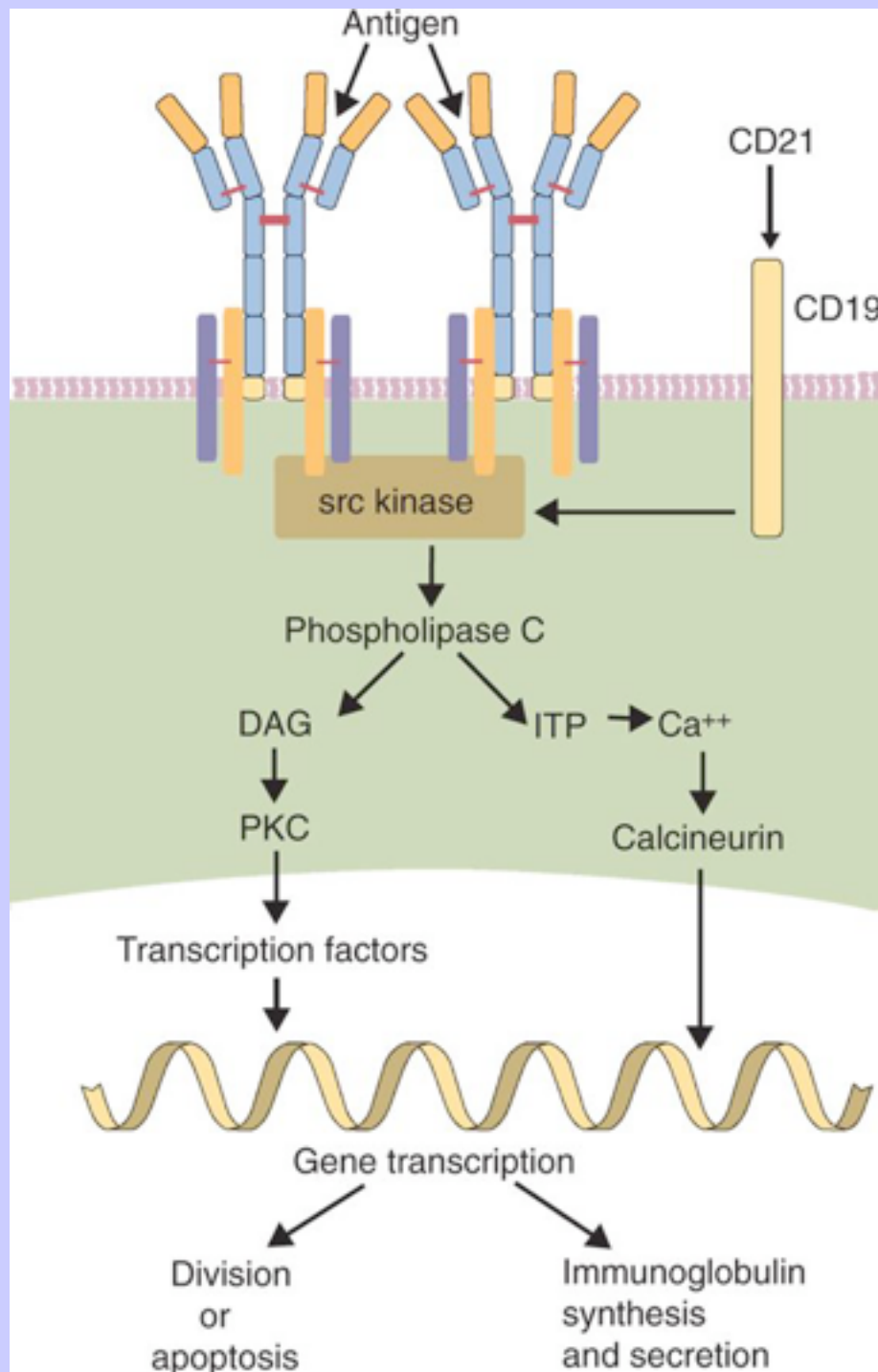


FIGURE 13-12 The differences in the time course of a T-dependent and T-independent antibody response. T-independent antigens cannot induce an immunoglobulin switch or immunological memory, as demonstrated by a secondary antibody response.

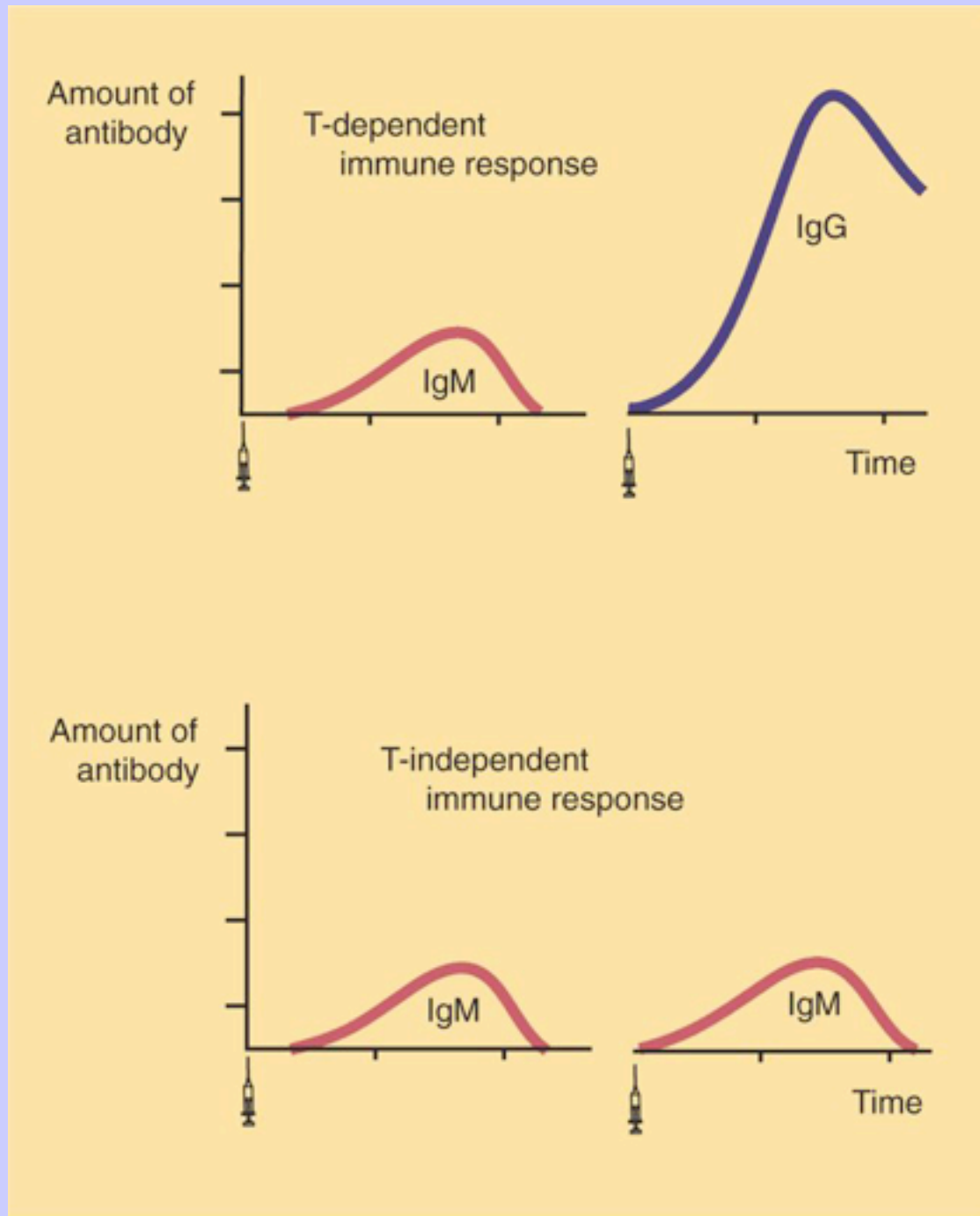
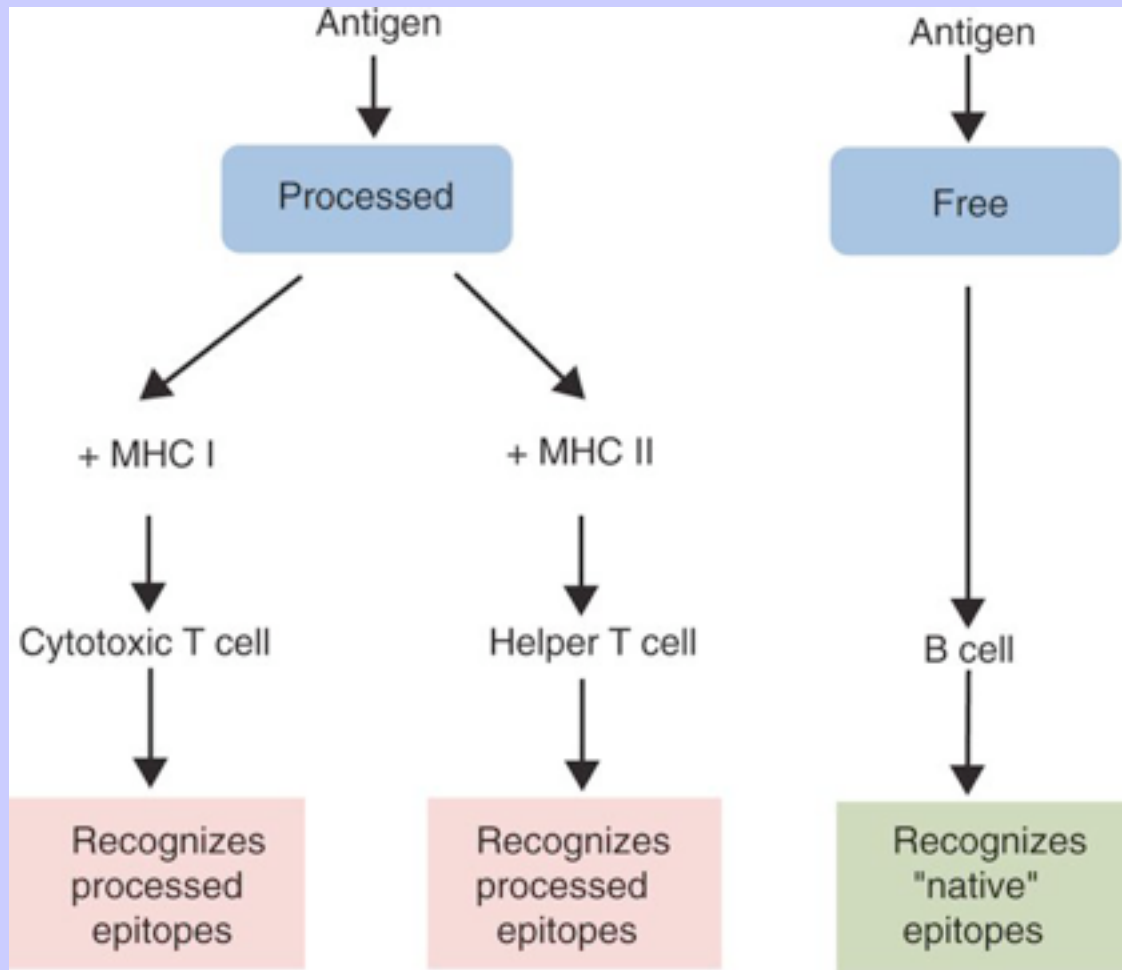


FIGURE 13-13 T cell antigen receptors (TCRs) and B cell antigen receptors (BCRs) recognize antigen in a very different fashion. Thus a BCR can bind to free, soluble antigen. A TCR, in contrast, can recognize only antigen processed and presented on a major histocompatibility complex (MHC) molecule.



antigens bind directly to B cell TLRs and cross-link several BCRs, thus providing a sufficient signal for B cell proliferation. Characteristically, T-independent antigens only trigger IgM responses and fail to generate memory cells ([Figure 13-12](#)). This is because they do not induce T cell co-stimulation and so cannot trigger the class switch.

It is appropriate to emphasize at this stage that the BCR has the same antigen-binding ability as do antibody molecules. Thus they can bind to free intact antigenic molecules in solution. This is very different from the α/β TCR that can bind and respond only to processed antigen bound to an MHC molecule ([Figure 13-13](#)). This difference in the antigen-recognizing ability of B and T cells is significant in that B cells can respond to a greater variety of antigens than can T cells.

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Likewise, antibodies are directed not against breakdown products of antigens but against intact antigen molecules. As a result, antigen-antibody interactions usually depend on maintenance of the three-dimensional conformation of the antigen. A good example of this is seen with tetanus toxoid. Antibodies raised against the intact molecule will bind only to the intact molecule and may be unable to bind to proteolytic fragments such as those produced by macrophage processing.

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13.5 CELLULAR RESPONSES

When antigen enters the body, it can stimulate a B cell only through its specific receptors. The term clonotype is used to describe a clone of B cells with a BCR capable of responding to a single epitope. A newborn animal has only a limited number of different clonotypes, but this number increases as the animal matures as a result of increased use of alternative sets of V genes and somatic hypermutation in these V genes (see [Chapter 15](#)). In an adult animal the number of B cells within a given clonotype varies as a result of exposure to different antigens over the animal's lifetime. Thus, as an individual ages, the most-used clonotypes will expand. For some rarely encountered antigens, there may be as few as 10 responsive cells in the spleen or bone marrow; for other, commonly encountered antigens, there may be as many as 10^4 responsive B cells.

In mice and probably most other mammals, each resting B cell carries both IgM and IgD BCRs on its surface, with about 10 times as many IgD molecules as IgM. These unstimulated B cells may secrete a small quantity of monomeric IgM. When antigen binds to the BCR in the presence of helper T cells with appropriate co-stimulation, signals are generated that result in increased expression of IgM, BCR, and MHC class II, as well as receptors for IL-4, IL-5, IL-6, tumor necrosis factor- α , and transforming growth factor- β (TGF- β). These signals start the process that leads to B cell division.

An appropriately stimulated B cell will enlarge and divide repeatedly. Some of its progeny begin to differentiate. They develop a rough endoplasmic reticulum, increase their rate of synthesis, and start to secrete large quantities of immunoglobulins. Within a few days, the cell switches from making IgM to making another immunoglobulin class. This switch occurs while a B cell is in the germinal center and leads to production of IgG, IgA, or IgE. The class switch results from deletion of unwanted heavy-chain genes and the joining of variable-region genes to the next available heavy chain genes (see [Chapter 14](#)). The specificity of the antibody produced remains unchanged. Class switching is controlled by IL-4, IFN- γ , and TGF- β . Thus IL-4 from Th2 cells directs mouse B cells to produce IgG1 and IgE, whereas it directs human B cells to produce IgG4 and IgE (see [Table 13-1](#)). IL-4 alone is insufficient for class switching, and additional signals are required to complete the process. In humans, the additional signals are provided through CD40 and CD154. IFN- γ from Th1 cells stimulates a switch to IgG2a and IgG3 in mouse B cells and effectively suppresses the effects of IL-4.

13.6 PLASMA CELLS

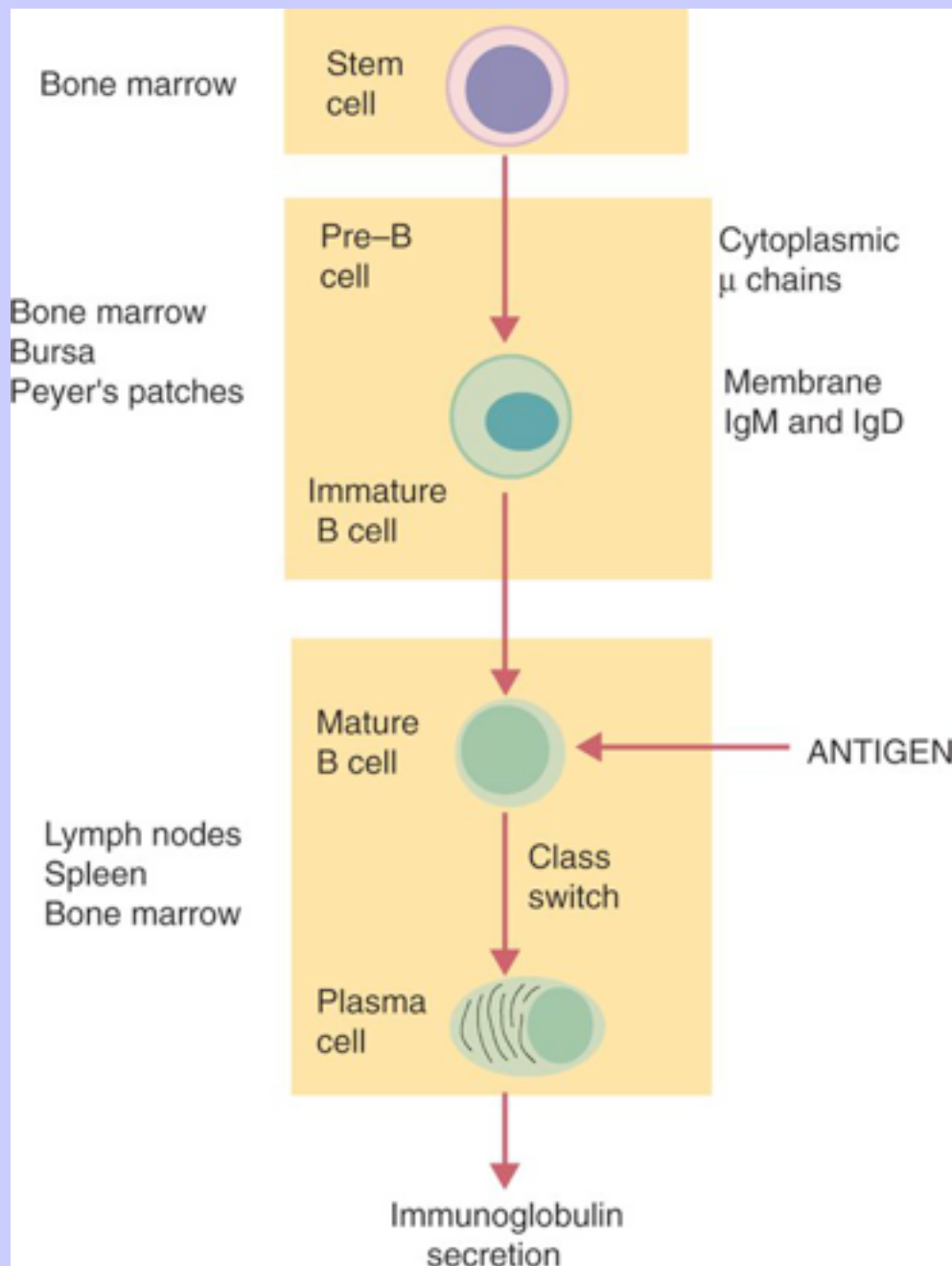
Plasma cells develop from antigen-stimulated B cells ([Figure 13-14](#)). Cells that are structurally intermediate among lymphocytes and plasma cells (plasmablasts) can be identified in the lymph node cortex and paracortex and in the marginal zone in the spleen. Fully developed plasma cells emigrate from these areas and are distributed throughout the body. They are found in greatest numbers in the spleen, in the medulla of lymph nodes, and in the bone marrow.

Plasma cells are ovoid cells, 8 to 9 μm in diameter ([Figure 13-15](#)). They have a round, eccentrically placed nucleus with unevenly distributed chromatin. As a result, the nucleus may resemble a clock face or cartwheel. Plasma cells have an extensive cytoplasm that is rich in rough endoplasmic reticulum and so stains strongly with basic dyes and

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pyronin. They have a large, pale-staining Golgi apparatus (Figures 13-16 and 13-17). Plasma cells can make and secrete up to 10,000

FIGURE 13-14 B cells originate in the bone marrow and proceed through a series of differentiation stages before becoming able to respond to antigen. When B cells respond to antigen, they respond by division and differentiation of their progeny into plasma cells.



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FIGURE 13-15 The structure of a typical plasma cell. The possession of an extensive rough endoplasmic reticulum is typical of a cell dedicated to the rapid production of large amounts of immunoglobulin.

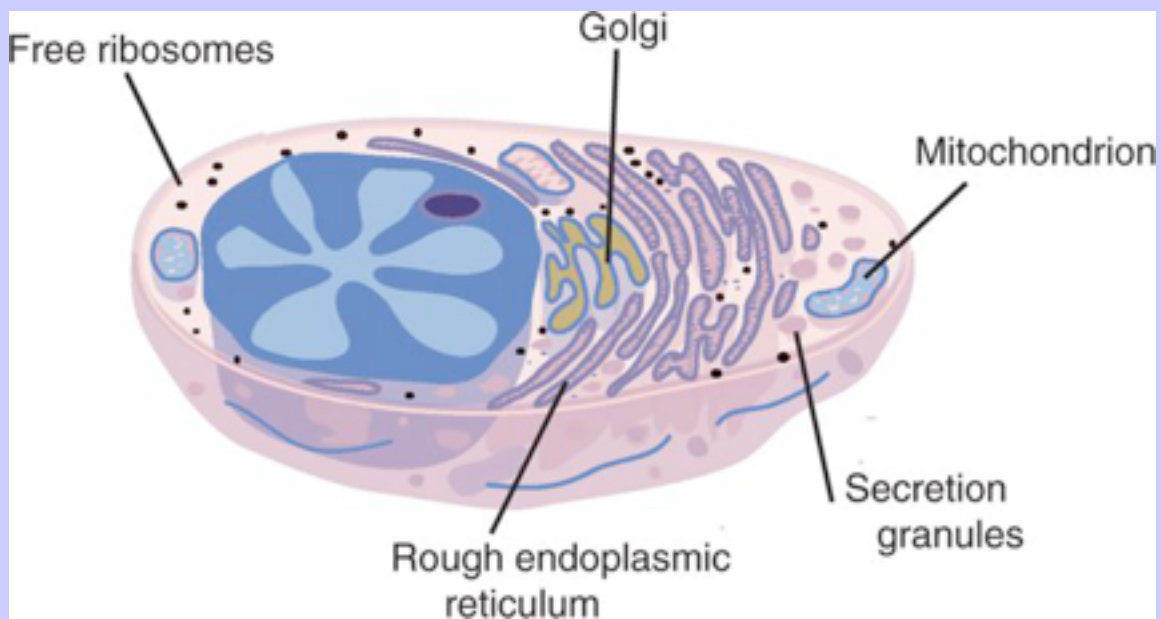


FIGURE 13-16 A transmission electron micrograph of a plasma cell from a rabbit. (Courtesy Dr. S. Linthicum.)

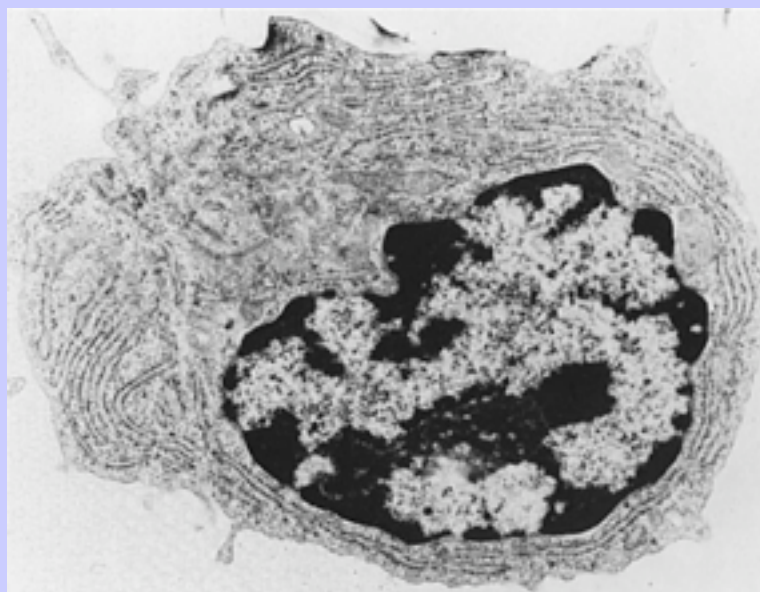
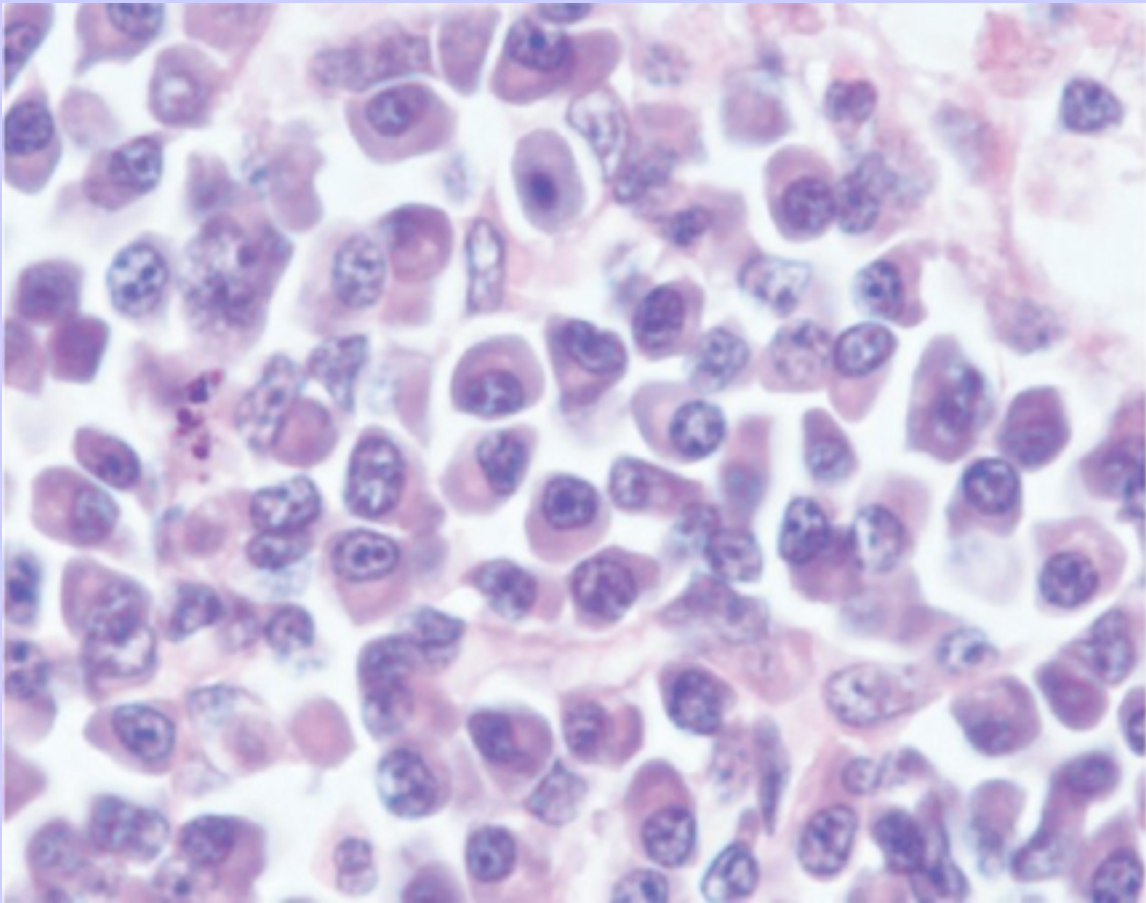


FIGURE 13-17 Plasma cells in the medulla of a dog lymph node. Their cytoplasm is rich in ribosomes and so stains intensely with pyronin, giving a dark red appearance. Original magnification $\times 450$. (From a specimen kindly provided by Drs. N. McArthur and L.C. Abbott.)



molecules of immunoglobulin per second. The immunoglobulin produced by a plasma cell is of identical specificity to the BCRs on its parent B cell.

13.7 MEMORY CELLS

One reason the primary immune response ends is that the responding B cells and plasma cells are simply removed by apoptosis. If all these cells died, however, immunological memory could not develop. Clearly some B cells must survive as memory cells. B cells are activated by antigen and helper T cells in the paracortex of lymph nodes. Most of these B cells differentiate into plasma cells and migrate to the bone marrow, but some memory precursors remain in the cortex, proliferate, and form germinal centers. These cells persist in the germinal center under the influence of programming and rescue signals. Thus memory cells are first screened for their ability to bind antigen. This induces CD154 on nearby T cells, which in turn facilitates memory B cell survival by promoting expression of *bcl-2*. *Bcl-2* protects against apoptosis and so allows a B cell to differentiate into a memory cell (see [Chapter 16](#)).

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Memory cells form a reserve of antigen-sensitive cells to be called on following subsequent exposure to an antigen. There are probably several types of memory cells. One population consists of small, long-lived resting cells with IgG BCR. These cells, unlike plasma cells, do not have a characteristic morphology but resemble other lymphocytes. Their prolonged survival does not depend on antigen contact. On exposure to antigen they proliferate and differentiate into plasma cells without undergoing further mutation. It has been calculated that in a secondary immune response, the clonal expansion of memory B cells results in eightfold to tenfold more plasma cells than does a primary immune response. A second type of memory cell population consists of large, dividing cells with IgM BCR. These cells persist within germinal centers, where their continued survival depends on exposure to antigen on follicular dendritic cells. In humans, memory B cell levels appear to be stable for up to 60 years postvaccination, indicating that they are under the control of very effective homeostatic mechanisms.

One feature of B cell memory is the prolonged production of antibodies over many months or years after immunization. Thus cats immunized with killed panleukopenia virus will continue to produce antibodies at low levels for many years. The source of these antibodies is believed to be long-lived plasma cells stimulated to secrete antibodies by exposure to PAMPs and by bystander T cell help.

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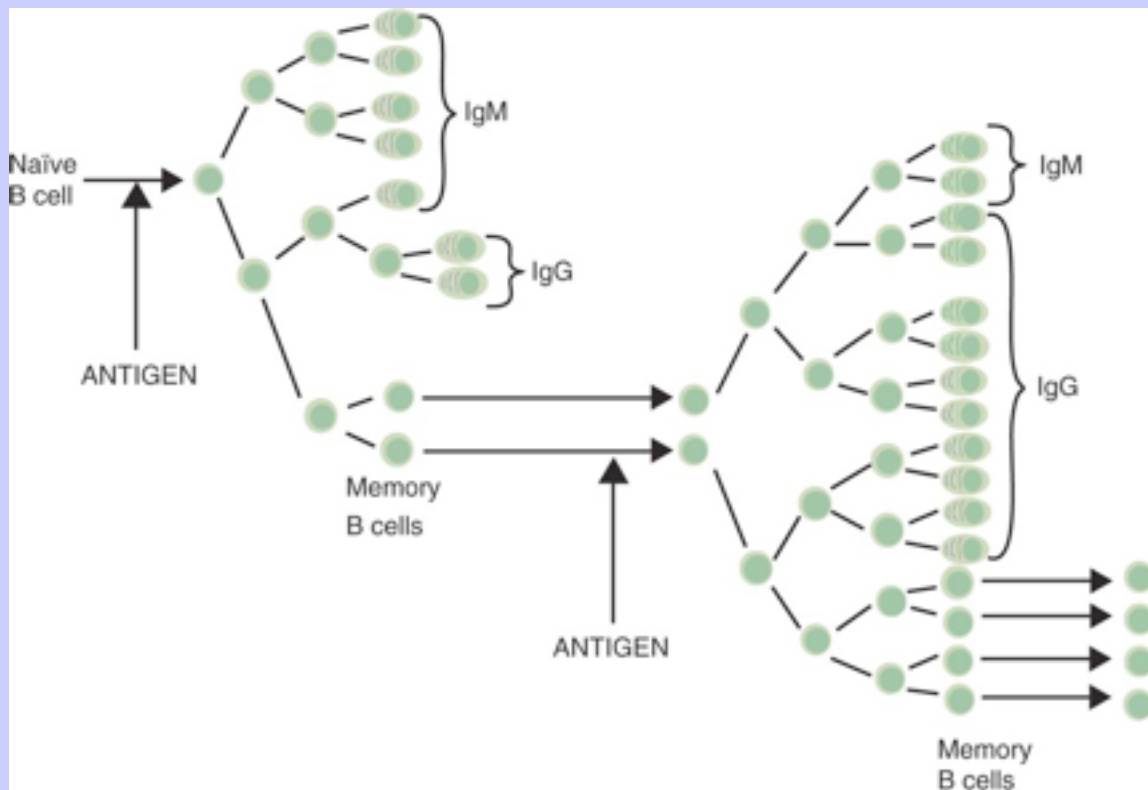
There are at least two populations of plasma cells: a short-lived population that lives for 1 to 2 weeks and produces large amounts of antibodies shortly after antigen exposure, and a long-lived population that can survive for months or years (perhaps as long as decades in humans). In humans these plasma cells have a half-life of 8 to 15 years and so produce antibodies for years after immunization. These antibodies will provide immediate immunity to microbial pathogens. The short-lived cells are found in spleen and lymph nodes soon after immunization. The long-lived plasma cells, in contrast, accumulate in the bone marrow. These long-lived plasma cells probably develop from a population of self-renewing, long-lived, slowly dividing memory B cells. These memory B cells require a functional BCR to survive, suggesting that constant low-affinity antigen binding keeps them alive.

If a second dose of antigen is given to a primed animal, it will encounter large numbers of memory B cells, which respond in the manner described previously for antigen-sensitive B cells ([Figure 13-18](#)). As a result, a secondary immune response is much greater than a primary immune response. The lag period is shorter, since more antibodies are produced and they can be detected earlier. IgG is also produced in preference to the IgM characteristic of the primary response.

13.8 GERMINAL CENTERS

As described in [Chapter 10](#), the development of germinal centers in the lymph nodes and spleen parallels the development of memory B cells. These germinal centers are sites where ([Figure 13-19](#)) antigen-driven cell proliferation, somatic hypermutation, and positive and negative selection of B cell populations occur. B cells stimulated by antigen and helper T cells migrate to a germinal center about 6 days after the response begins. There they divide rapidly. These large dividing cells give a germinal center its pale appearance in histological sections. During this rapid B cell division, the BCR V region genes mutate at a rate of about one mutation per division. This somatic mutation generates large numbers of B cells whose BCRs differ from those of the parent cell. Once these cells have been clonally expanded, a process that takes 10 to 20 days, they migrate to the periphery of the germinal center, where they encounter antigen on dendritic cells. Follicular dendritic cells in germinal centers trap antigen very effectively. Because of mutation, some of the germinal center B cells bind this antigen with greater affinity, but many, perhaps the great majority, bind the antigen less strongly. If mutation has resulted in greater affinity of the BCR for the antigen, then B cells with these receptors will divide further and leave the center to form either plasma cells or memory cells. However, the majority of mutated BCRs show reduced antigen binding. The cells with these receptors

FIGURE 13-18 The time course of a B cell response and the cellular events that accompany it. Note how some immunoglobulin G (*IgG*) is made in the primary immune response, whereas a small amount of *IgM* is made in a secondary immune response.



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FIGURE 13-19 B cells in the germinal center undergo somatic mutation as they respond to antigen presented by dendritic cells. If the mutation enables them to bind antigen more strongly, they will be stimulated to grow still further. If, on the other hand, the mutation reduces their antigen-binding ability, they will undergo apoptosis.

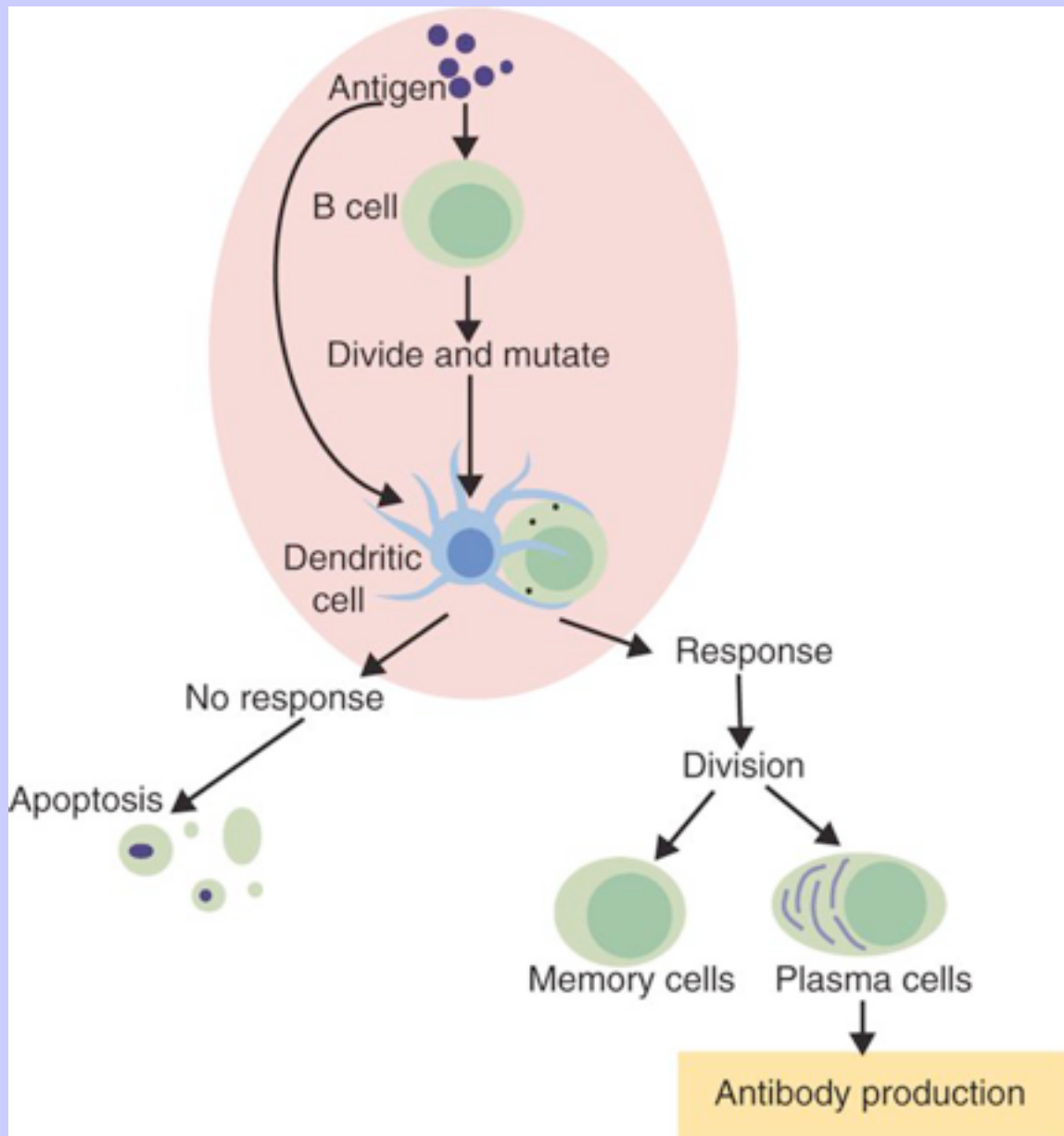
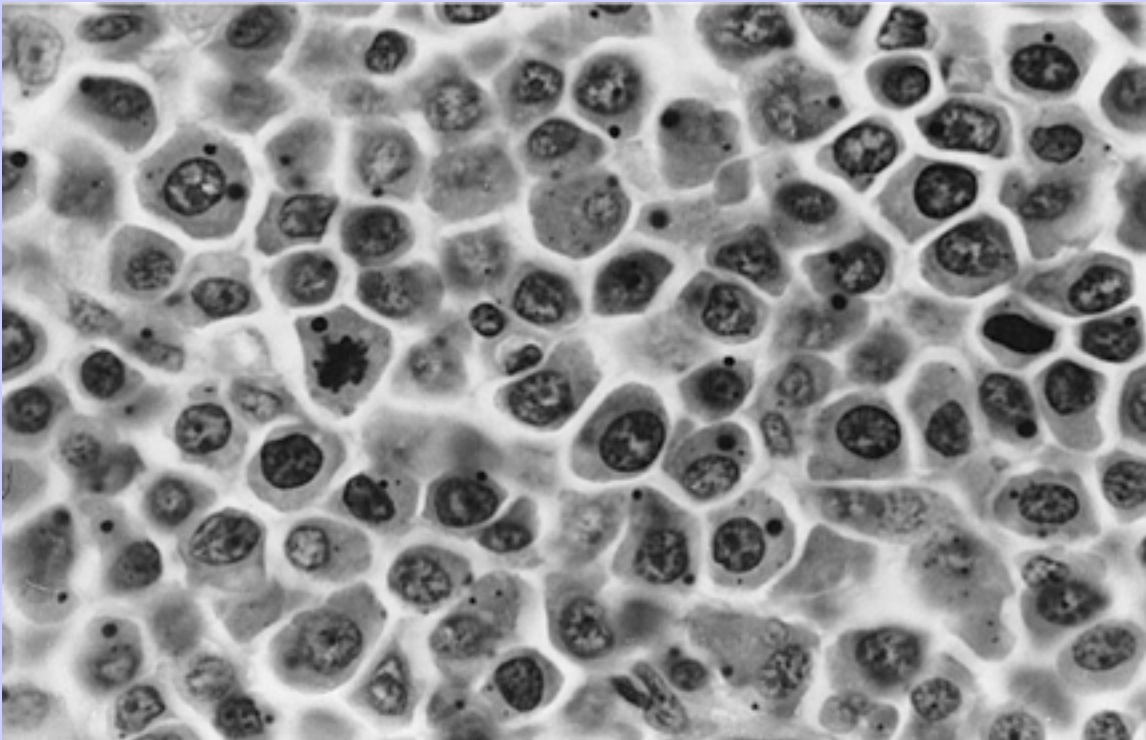


FIGURE 13-20 A section of myeloma tumor mass in a dog. Original magnification $\times 900$. The cells are clearly plasma cells. (From a specimen kindly provided by Dr. R.G. Thompson.)



undergo apoptosis, and their remains are removed by macrophages. Thus the B cell population that emerges from a germinal center is very different from the population of cells that entered it. In addition to mutation of BCR V genes, BCRs undergo the immunoglobulin class switch within germinal centers.

13.8.1 B Cell Subpopulations

There are two distinct subpopulations of B cells that develop from different precursor stem cells. They are called B1 and B2 cells. B2 cells are the conventional B cells that are central to acquired antibody responses and are discussed in this chapter and throughout the book. These B2 cells appear late in neonatal life and are the predominant population in adult bone marrow. They produce most of the body's IgG.

B1 cells, in contrast, originate from stem cells in the fetal liver or omentum rather than the bone marrow. There are two subpopulations of B1 cells termed B1a and B1b (in mice). B1a cells develop exclusively in the neonate, are self-replenishing, and are responsible for most "natural" IgM in serum. They participate in innate immunity. B1a cells express CD5, an adhesion and receptor molecule. (CD5 is the receptor for CD72.) They recognize common bacterial molecules such as phosphoryl choline, as well molecules such as phosphatidyl choline, immunoglobulins, and DNA. They produce antibodies in a T-independent manner. B1a cells also differ from conventional B2 cells in that they are found in the peritoneal and pleural cavities of rodents and have the

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potential to renew themselves. B1b cells are distinguished from B1a cells by lacking CD5. They are, however, required for protection against several parasites and bacteria. B1b cells are produced throughout adult life.

Many of the IgA-producing cells in the intestine originate from B1 cells. B1 cells have been identified in humans, mice, rabbits, guinea pigs, pigs, sheep, and cattle.

13.9 MYELOMAS

Malignant transformation of a single B cell may give rise to the development of a clone of immunoglobulin-producing tumor cells. The structure of these cells varies, but they are usually recognizable as plasma cells ([Figure 13-20](#)). Plasma cell tumors are called myelomas or plasmacytomas. Because myelomas arise from a single precursor cell or clone, they secrete a homogeneous immunoglobulin called a myeloma protein. On serum electrophoresis, this homogeneous myeloma protein will appear as a sharp, well-defined peak. This is called a monoclonal gammopathy ([Figure 13-21](#)).

Myeloma proteins may belong to any immunoglobulin class. For example, IgG, IgA, and IgM myelomas have been reported in the dog. In humans, in addition to myelomas of the major immunoglobulin classes, rare cases of IgD and IgE myelomas have also been described. The prevalence of the various immunoglobulin classes in myeloma proteins correlates well with their relative quantities in normal serum, indicating that the tumor arises as a result of a random mutation

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FIGURE 13-21 Serum electrophoretic patterns showing the normal pattern and the characteristic features of monoclonal and polyclonal gammopathies. The monoclonal antibody spike reflects the production of large amounts of homogenous immunoglobulins. Monoclonal gammopathies commonly result from the presence of a myeloma. The arrow denotes the direction of migration.

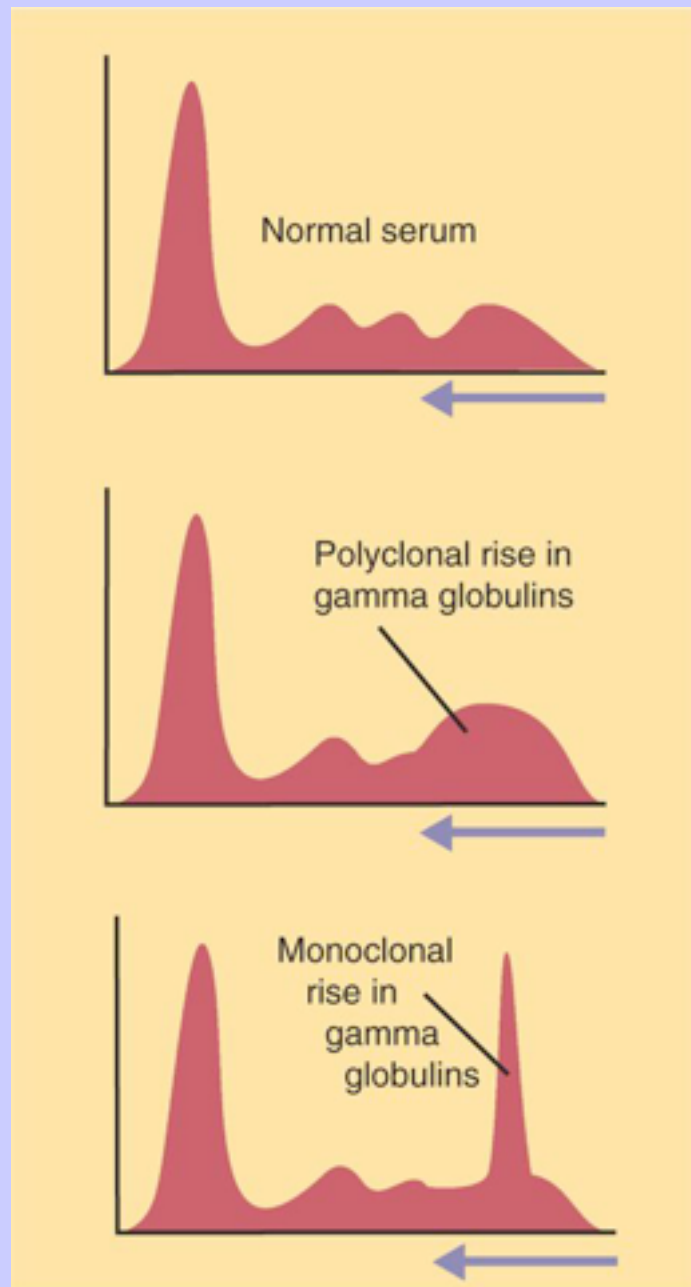


FIGURE 13-22 A radiograph of a dog, showing the round, radiolucent areas where bone has been eroded by the presence of a myeloma. (Courtesy Dr. Claudia Barton.)



in a single B cell. Light chain disease is a myeloma in which light chains alone are produced or the production of light chains is greatly in excess of the production of heavy chains. Similarly, there is a very rare form of myeloma in which Fc fragments alone are produced. This condition is erroneously termed heavy chain disease.

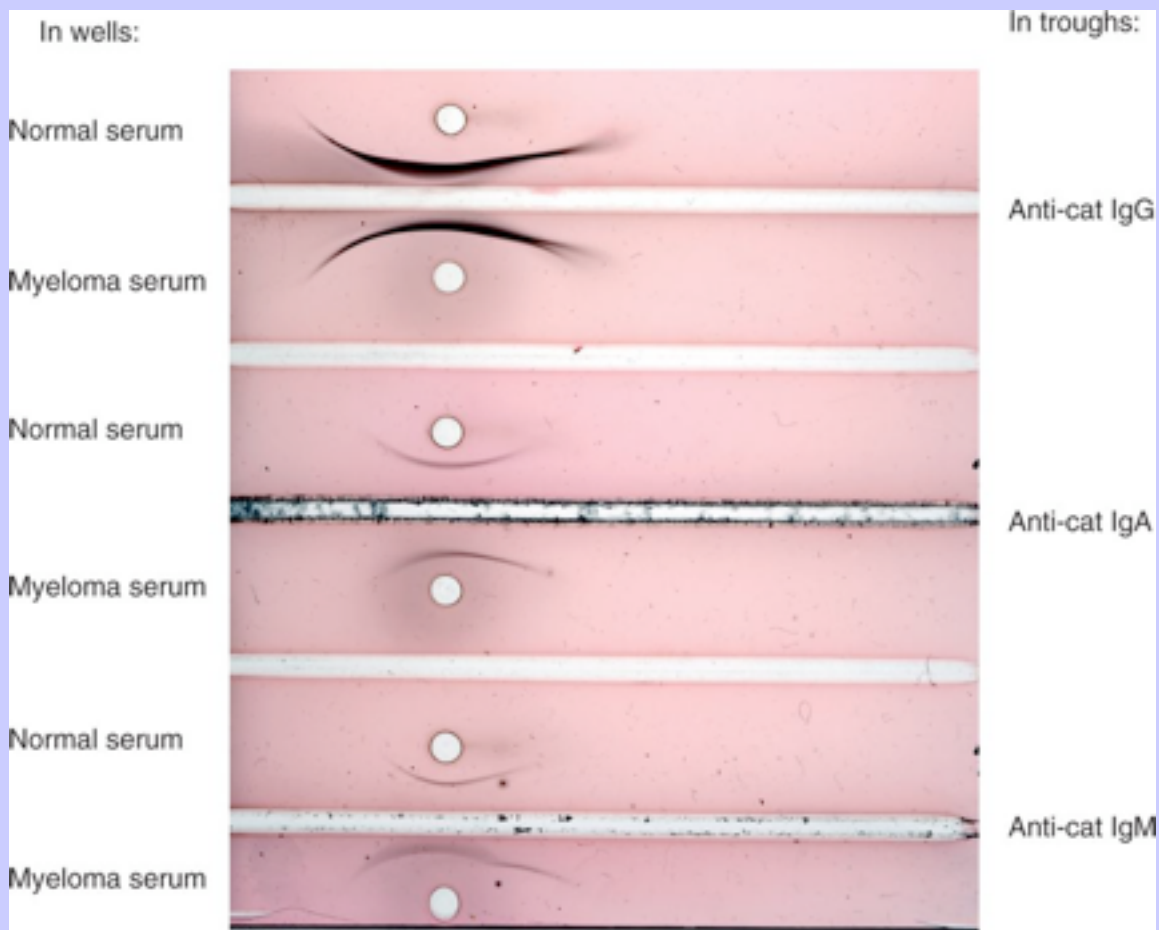
Myelomas have been described in humans, mice, dogs, cats, horses, cows, pigs, ferrets, and rabbits. They account for less than 1% of all canine tumors, and they are considerably rarer in the other domestic species. The clinical

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presentation of myelomas is highly variable and differs between species. There are, however, several key manifestations, including bleeding disorders, hyperviscosity, renal failure, and hypercalcemia. Other symptoms include lethargy, recurrent infections, anemia, lameness, bone fracture, and neurological signs, including dementia and peripheral neuropathy. The most common clinical manifestation in dogs is excessive bleeding as a result of a thrombocytopenia and a loss of clotting components as they bind to myeloma proteins. The presence in serum of abnormally large quantities of immunoglobulins results in a hyperviscosity syndrome, which is especially severe in animals with IgM myelomas (macroglobulinemia). As a result of the increase in blood viscosity, the heart must work harder and congestive heart failure, retinopathy, and neurological signs may result. Because myeloma cells stimulate osteoclast activity, the presence of tumors in bone marrow may lead to severe bone destruction. Multiple radiolucent osteolytic lesions and diffuse osteoporosis develop and are readily seen by radiography ([Figure 13-22](#)). These lesions result in pathological fractures. Light chains, being relatively small, are excreted in the urine. Unfortunately, they are toxic for renal tubular cells and, as a result, may cause renal failure. The light chains may be detected by electrophoresis of concentrated urine or, in some cases, by heating the urine. Light chains precipitate when heated to 60° C but redissolve as the temperature is raised to 80° C. Proteins possessing this curious property are called Bence-Jones proteins, and their presence in urine suggests a myeloma. They are seen in about 40% of canine cases. Nonsecretory myelomas are occasionally diagnosed in dogs.

Because of the overwhelming commitment of the body's immune resources to the production of neoplastic plasma cells, as well as the replacement of normal marrow tissue by tumor cells and the negative feedback induced by elevated serum immunoglobulins, animals with myelomas are immunosuppressed and anemic. In humans, renal failure and overwhelming

FIGURE 13-23 Immuno-electrophoresis of cat serum. Note that the line of precipitate formed by the reaction between anti-cat immunoglobulin M (IgM) and the myeloma serum is distorted (*bottom well*). The line is much thicker than the control, and it forms two distinct joined arcs as a result of the presence of a monoclonal IgM myeloma protein. (Details of this technique can be found in [Chapter 38](#).) (Courtesy Dr. G. Elissalde.)



infection are the most common causes of death in myeloma patients.

Animals with myelomas characteristically have a monoclonal gammopathy that can be identified by serum electrophoresis. The class of immunoglobulin involved can be identified by immunoelectrophoresis ([Figure 13-23](#)), and it may be measured by radial immunodiffusion (see [Chapter 38](#)).

Affected animals should receive supportive therapy to relieve their immediate clinical problems. Antibiotics can be used to control secondary infections, and fluid therapy should be administered to combat the dehydration resulting from renal failure. Steroids and diuretics may assist in promoting calcium excretion. The serum hyperviscosity may

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be reduced by plasmapheresis to remove the myeloma protein. The tumor itself can be treated with specific chemotherapy. The drug of choice is melphalan, an alkylating agent. Prednisone may be used in association with melphalan. In unresponsive cases cyclophosphamide or thalidomide may be employed.

Sometimes, in clinically normal humans, dogs, and horses, a monoclonal gammopathy may develop that is not due to a myeloma. These monoclonal antibodies are usually an inadvertent finding on serum electrophoresis, and their origin is unclear. They may disappear spontaneously within a short period, or they may persist for many years. On necropsy affected animals may show abnormally large numbers of plasma cells in their internal organs.

In contrast to monoclonal gammopathies, which are usually produced by a myeloma, polyclonal gammopathies are observed in a wide variety of patho-logical conditions. Polyclonal gammopathies are characterized by an increase in all immunoglobulins as a result of excessive activity of many different clones of plasma cells. The condition that most resembles a myeloma is Aleutian disease in mink (see [Chapter 23](#)). Animals infected by the Aleutian disease virus show, in the progressive form of the disease, marked plasmacytosis and lymphocyte infiltration of many organs and tissues, as well as polyclonal (occasionally monoclonal) gammopathy. As a result of the elevated immunoglobulin levels, affected mink experience a hyperviscosity syndrome and are severely immunosuppressed.

Other causes of polyclonal gammopathy include autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, and myasthenia gravis (see [Chapter 33](#)), as well as certain infections such as tropical pancytopenia of dogs due to *Ehrlichia canis*, African trypanosomiasis, and chronic bacterial infections such as pyometra and pyoderma. In horses heavily parasitized with *Strongylus vulgaris*, polyclonal IgG3 levels rise significantly. Polyclonal gammopathy also occurs in virus diseases such as feline infectious peritonitis and African swine fever and in diseases in which there is extensive liver damage.

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13.10 HYBRIDOMAS

The plasma cells in myelomas become neoplastic in an entirely random manner, so that the immunoglobulins that they secrete are not usually directed against any antigen of practical importance. Nevertheless, myeloma cells can be grown in tissue culture, where they survive indefinitely. It is highly desirable to be able to establish a system to obtain large quantities of absolutely pure, specific immunoglobulins directed against an antigen of interest. This can be done by fusing a normal plasma cell, marking the antibody of interest, with a myeloma cell able to grow in tissue culture. The resulting mixed cell is called a hybridoma.

The first stage in making a hybridoma is to generate antibody-producing plasma cells ([Figure 13-24](#)). This is done by immunizing a mouse against the antigen of interest and repeating the process several times to ensure that a good antibody response is mounted. Two to 4 days after the antigen is administered, the spleen is removed and broken up to form a cell suspension. These spleen cells are suspended in culture medium, together with cultured mouse myeloma cells. Generally, myeloma cells that do not secrete immunoglobulins are used, since this simplifies purification later on. Polyethylene glycol is added to the mixture. This compound induces many of the cells to fuse (although it takes about 200,000 spleen cells on average to form a viable hybrid with one myeloma cell). If the fused cell mixture is cultured for several days, any unfused spleen cells will die. The myeloma cells would normally survive, but they are eliminated by a simple trick.

There are three biosynthetic pathways by which cells can synthesize nucleotides and therefore nucleic

FIGURE 13-24 A schematic diagram showing the method of production of monoclonal antibodies. Antibody-producing plasma cells are fused with myeloma cells. The resulting hybridoma cells are cultured, cloned, and selected so that they produce antibodies against the antigen of interest.

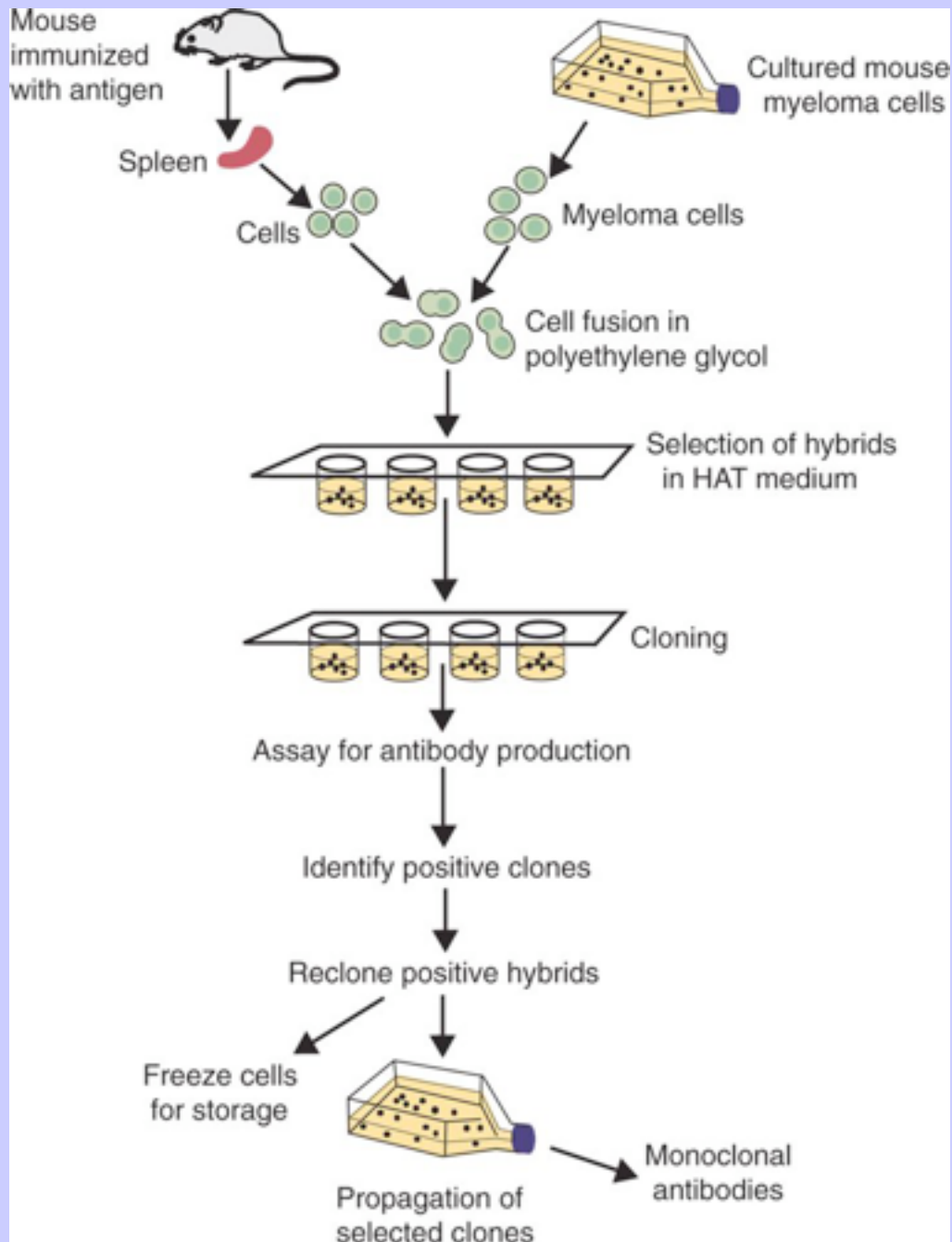
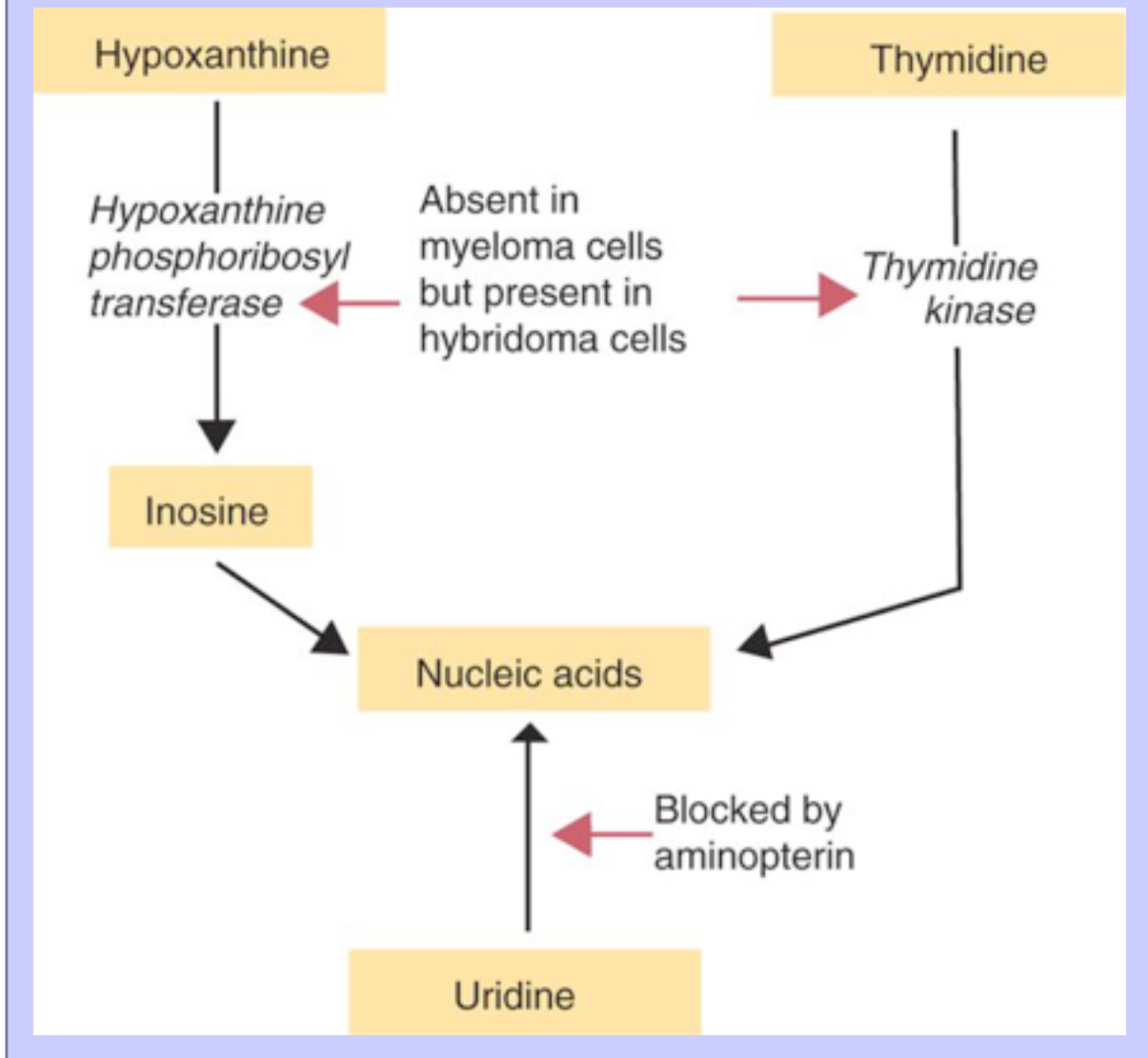


FIGURE 13-25 The pathways of purine synthesis and the mechanism of action of hypoxanthine-aminopterin-thymidine medium.



acids. The myeloma cells are selected so that they lack two enzymes: hypoxanthine phosphoribosyl transferase and thymidine kinase. As a result, they cannot use either thymidine or hypoxanthine and are obliged to use an alternative biosynthetic pathway to convert uridine to nucleotides. The fused cell mixture is therefore grown in a culture containing three compounds: hypoxanthine, aminopterin, and thymidine (known as HAT medium). Aminopterin is a drug that prevents cells from making their own nucleotides from uridine. Since the myeloma cells cannot use hypoxanthine or thymidine and the aminopterin stops them from using the alternative synthetic pathway, they cannot make nucleic acids and soon die ([Figure 13-25](#)). Hybrid cells made from a myeloma and a normal cell will grow, since they possess the critical enzymes. The hybridomas divide rapidly in the HAT medium, doubling their numbers every 24 to 48 hours. On average, about 300 to 500 different hybrids can be isolated from a mouse spleen, although not all will make antibodies of interest.

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If a mixture of cells from a fusion experiment is cultured in wells on a plate with about 50,000 myeloma cells per well, it is usual to obtain about one hybrid in every three wells. After culturing for 2 to 4 weeks, the growing cells can be seen and the supernatant fluid can be screened for the presence of antibodies. It is essential to use a sensitive assay at this time. Radioimmunoassays or enzyme-linked immunosorbent assays are preferred (see [Chapter 38](#)). Clones that produce the desired antibody are grown in mass culture and recloned to eliminate non-antibody-producing hybrids.

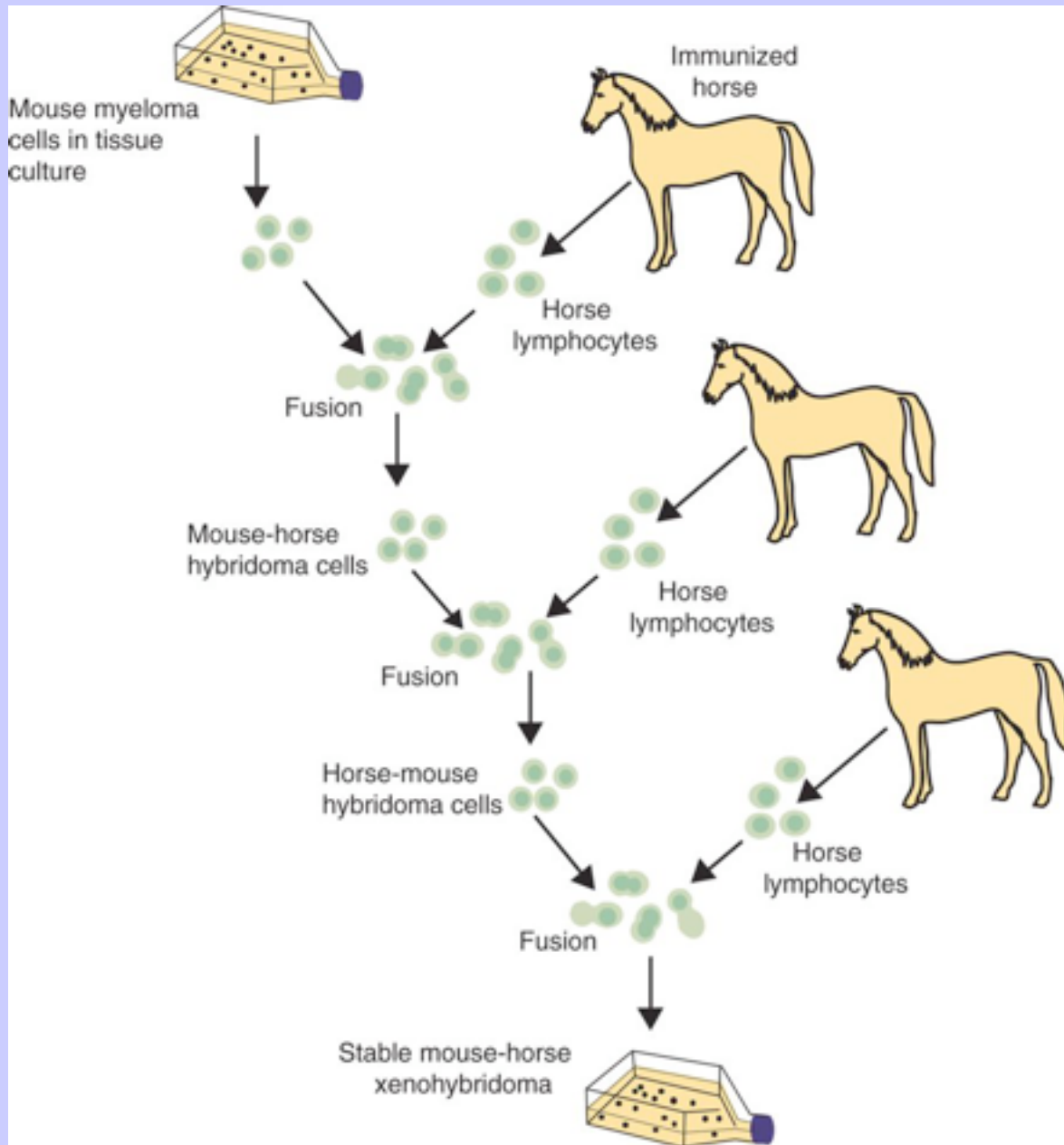
Unfortunately, antibody-producing clones tend to lose this ability after being cultured for several months. Thus it is usual to make large stocks of hybridoma cells and store them frozen in small aliquots. These can then be thawed as required and grown up in bulk culture. Alternatively, the hybridoma cells can be injected intraperitoneally into mice. Since they are tumor cells, the hybridomas grow rapidly and provoke the effusion of a large volume of fluid into the mouse peritoneal cavity. This fluid is rich in monoclonal antibody and can be readily harvested.

Although the classical methods of making hybridomas produce only mouse immunoglobulins, it is possible to produce monoclonal antibodies from cells of other mammalian species. For example, it is possible to make bovine hybridomas by fusing bovine B cells with a cultured bovine lymphoblastoid cell line. It is generally easier, however, to make hybridomas by fusing B cells from the species under study with mouse myeloma cells. These xenohybridomas or hetero-hybridomas are made as described previously, but the source of the antibody-producing cells is a species other than a mouse. Thus equine xenohybridomas can be produced by fusing antibody-producing equine spleen cells with mouse myeloma cells. The resulting interspecific hybridomas may secrete equine monoclonal antibodies. Unfortunately, these xenohybridoma cells are unstable and tend to lose the nonmurine chromosomes as they divide. As a result, they may cease immunoglobulin synthesis prematurely. Improved stability can be achieved by first growing the xenohybridoma cells in the presence of 8-azaguanine to select for aminopterin sensitivity. These xenohybridomas are then used as fusion partners with lymphocytes from immunized animals of the correct species ([Figure 13-26](#)). The resulting secondary xenohybridomas may be further selected and used as fusion partners to produce tertiary xenohybridomas.

Monoclonal antibodies have become the preferred source of antibodies for much immunological research. They are absolutely specific for single epitopes and are available in large numbers. Because of their purity, they can function as standard chemical reagents. Monoclonal antibodies are rapidly being incorporated into clinical diagnostic techniques in which large quantities of antibodies of consistent quality are required. Although mouse cells have been the preferred source, recent experimental studies have shown that cattle and goats can be genetically engineered to produce monoclonal antibodies in their milk. It has even proved possible to incorporate antibody genes into plants such as soy, corn, or tobacco. These “plantibodies” are produced in very large quantities and, although heavily glycosylated, appear to be functional.

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FIGURE 13-26 A schematic diagram showing one method of making xenohybridomas, in this case using horse cells.



13.11 SOURCES OF ADDITIONAL INFORMATION

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¹⁴ CHAPTER 14 Antibodies: Soluble Antigen Receptors

^{14.1} KEY POINTS

- There are five classes of immunoglobulins in mammals: IgG, IgM, IgA, IgE, and IgD. All originate as B cell antigen receptors shed into body fluids.
- IgG is the predominant immunoglobulin in serum and is mainly responsible for systemic defense.
- IgM is a very large immunoglobulin produced mainly during a primary immune response.
- IgA is the immunoglobulin produced on body surfaces. It is responsible for the defense of the intestinal and respiratory tracts.
- IgE is found in very small quantities in serum and is responsible for immunity to parasitic worms and for allergies.
- IgD is found on the surface of immature lymphocytes. Its function is unknown.

The properties of the B cell antigen receptors (BCRs) are discussed in [Chapter 13](#). These receptors are, however, not restricted to the B cell surface. Once a B cell response is triggered, the receptors are shed into the surrounding fluid, where they act as antibodies. These antibodies bind to specific antigens and hasten their destruction or elimination. Antibodies are found in many body fluids but are present in highest concentrations and are most easily obtained from blood serum. Antibodies have to defend an animal against a variety of microbes, including bacteria, viruses, and protozoa. They also must act in several different environments—for example, in blood, milk, and body surfaces. It is not surprising, therefore, that several different immunoglobulin classes exist. Each class is optimized for action in a specific environment: for instance, IgA protects body surfaces. Immunoglobulins may also be optimized for activity against a specific group of pathogens. For example, IgE is especially effective against parasitic worms.

^{14.2} IMMUNOGLOBULINS

Antibody molecules are glycoproteins called immunoglobulins (Ig). The term immunoglobulin is used to describe all soluble BCRs. There are five different classes (or isotypes) of immunoglobulins, which differ in their use of heavy chains. The class found in highest

FIGURE 14-1 The electrophoresis of a protein mixture on a strip of paper or other support. The support bridges two buffer baths, and an electrical potential is applied across them.

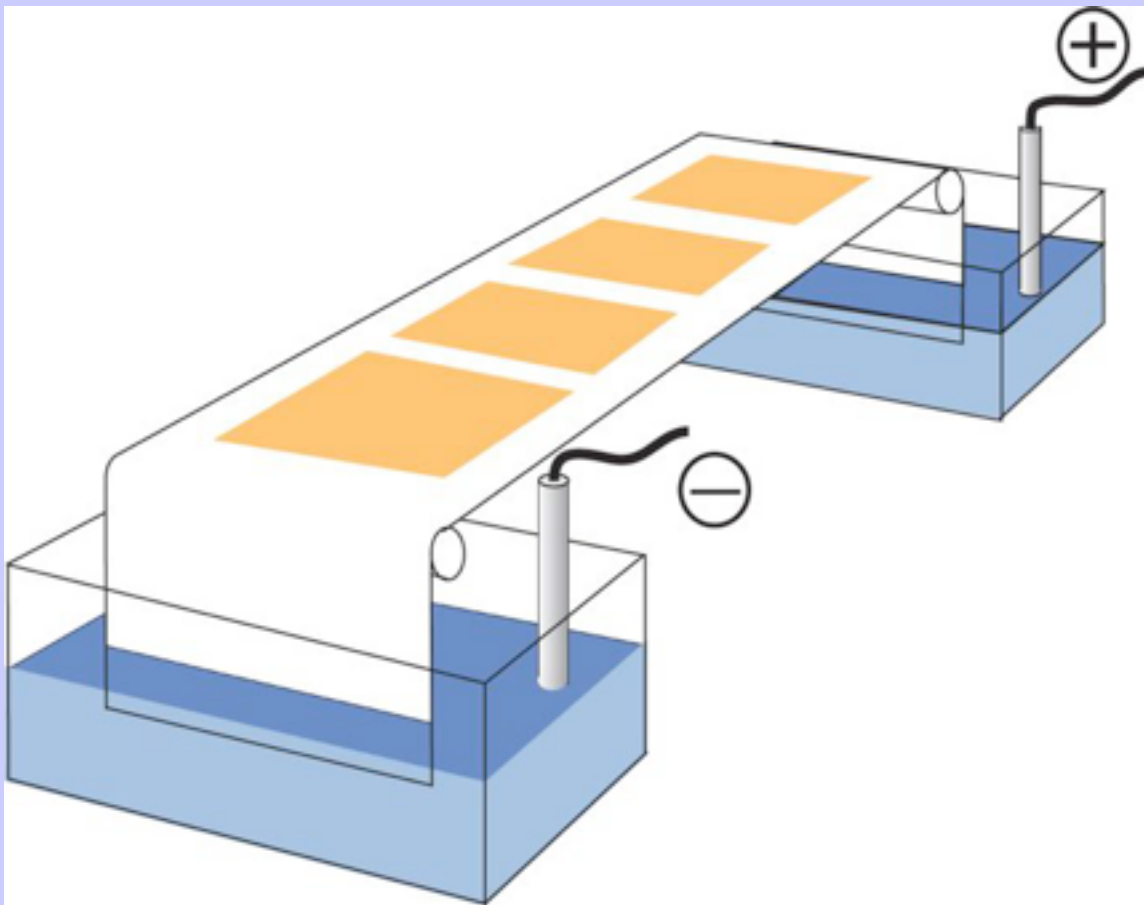
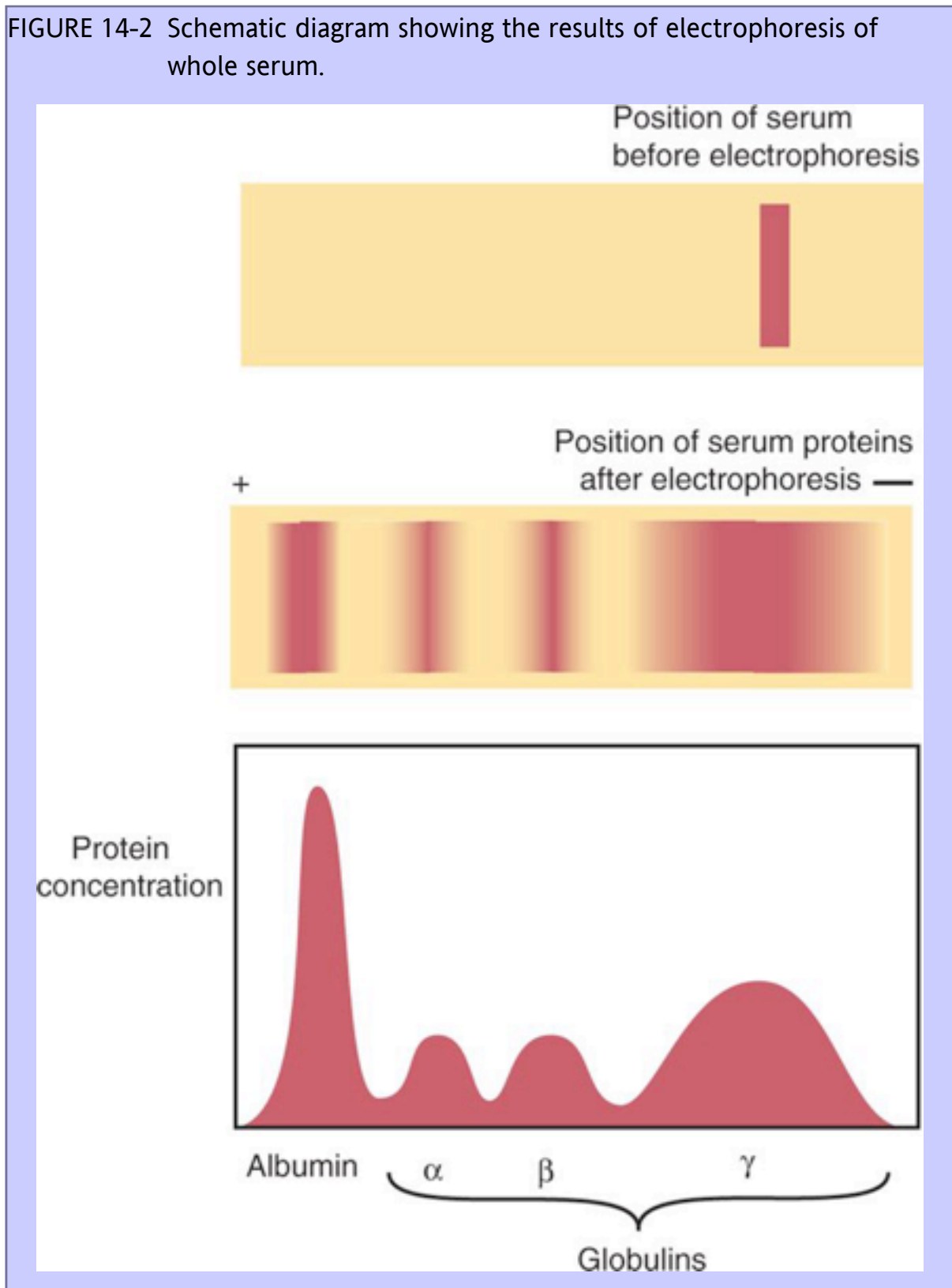


FIGURE 14-2 Schematic diagram showing the results of electrophoresis of whole serum.



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concentrations in serum is called immunoglobulin G (IgG). The class with the second-highest serum concentration (in most mammals) is immunoglobulin M (IgM). The third-highest concentration in most mammals is immunoglobulin A (IgA). IgA is, however, the predominant immunoglobulin in secretions such as saliva, milk, and intestinal fluid. Immunoglobulin D (IgD) is primarily a BCR and so is rarely encountered in body fluids. Immunoglobulin E (IgE) is found in very low concentrations in serum and mediates allergic reactions. The characteristics of each of these classes are shown in [Table 14-1](#).

Table 14-1 Major Immunoglobulin Classes in the Domestic Mammals

Property	Immunoglobulin Class				
	IgM	IgG	IgA	IgE	IgD
Molecular weight	900,000	180,000	360,000	200,000	180,000
Subunits	5	1	2	1	1
Heavy chain	μ	γ	α	ε	δ
Largely synthesized in:	Spleen and lymph nodes	Spleen and lymph nodes	Intestinal and respiratory tracts	Intestinal and respiratory tracts	Spleen and lymph nodes

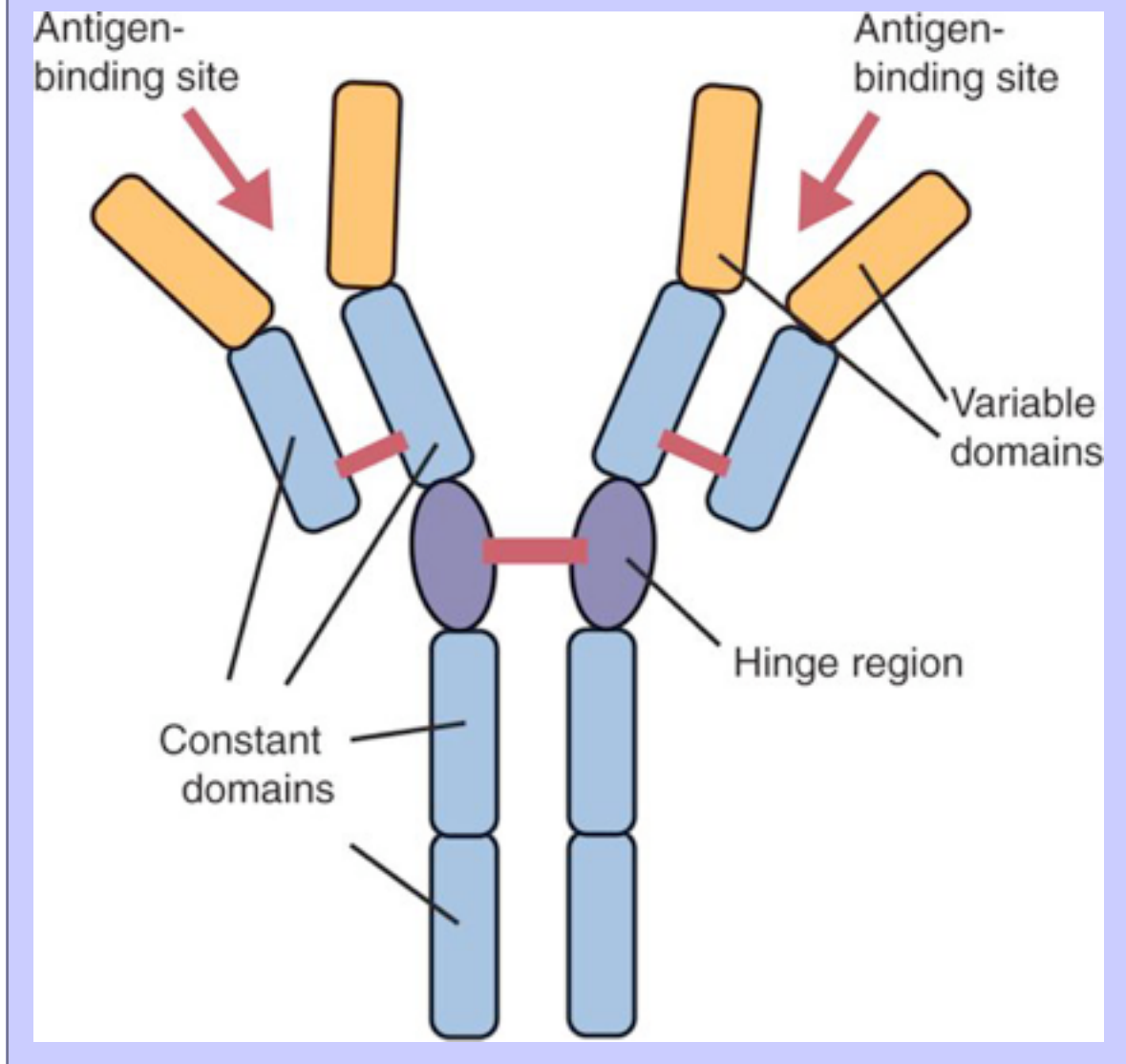
When serum is subjected to electrophoresis, its proteins separate into four major fractions ([Figure 14-1](#)). The most negatively charged fraction consists of a single, homogeneous protein called serum albumin. The other three major fractions contain protein mixtures classified as α, β, and γ globulins, according to their electrophoretic mobility ([Figure 14-2](#)). Most immunoglobulins are found in the γ globulins, although IgM migrates among the β globulins.

14.3 IMMUNOGLOBULIN CLASSES

14.3.1 Immunoglobulin G

IgG is made and secreted by plasma cells in the spleen, lymph nodes, and bone marrow. It is the immunoglobulin found in highest concentration in the blood ([Table 14-2](#)) and for this reason plays the major role in antibody-mediated defense mechanisms. It has a molecular weight of about 180 kDa and a typical BCR structure with two identical light chains and two identical γ heavy chains ([Figure 14-3](#)). Its light chains may be of the κ or λ type. Because it is the smallest of the

FIGURE 14-3 The structure of IgG, the prototypical immunoglobulin molecule. Compare this with [Chapter 13, Figure 13-6](#), a typical B cell antigen receptor.



immunoglobulin molecules, IgG can escape from blood vessels more easily than can the others. This is especially important in inflammation, where increased vascular permeability allows IgG to participate in the defense of tissues and body surfaces. IgG binds to specific antigens such as those found on bacterial surfaces. Binding of these antibody molecules to bacterial surfaces can cause clumping (agglutination) and opsonization. IgG antibodies can activate the classical complement pathway only when sufficient molecules have accumulated in a correct configuration on the antigenic surface (see [Chapter 5](#)).

Table 14-2 Serum Immunoglobulin Levels in the Domestic Animals and Human

Species	Immunoglobulin Levels (mg/dl)			
	IgG	IgM	IgA	IgE
Horse	1000–1500	100–200	60–350	
Cattle [*]	1700–2700	250–400	10–50	
Sheep	1700–2000	150–250	10–50	
Pig	1700–2900	100–500	50–500	
Dog	1000–2000	70–270	20–150	1–7
Cat [†]	400–2000	30–150	30–150	
Chicken	300–700	120–250	30–60	
Human	800–1600	50–200	150–400	0.002–0.05

* Cattle show significant seasonal differences in serum immunoglobulin levels.

† Immunoglobulin levels in specific-pathogen-free cats are approximately half those in pet cats.

14.3.2 Immunoglobulin M

IgM is also produced by plasma cells in the spleen, lymph nodes, and bone marrow. It occurs in the second-highest concentration after IgG in most mammalian serum. While on the B cell surface and acting as a BCR, IgM is a 180-kDa immunoglobulin monomer. However, the secreted form of IgM consists of 5 (occasionally 6) 180 kDa subunits linked by disulfide bonds in a circular fashion. Its total molecular weight is 900 kDa (Figure 14-4). A small polypeptide called the J chain (15 kDa) joins two of the units to complete the circle.

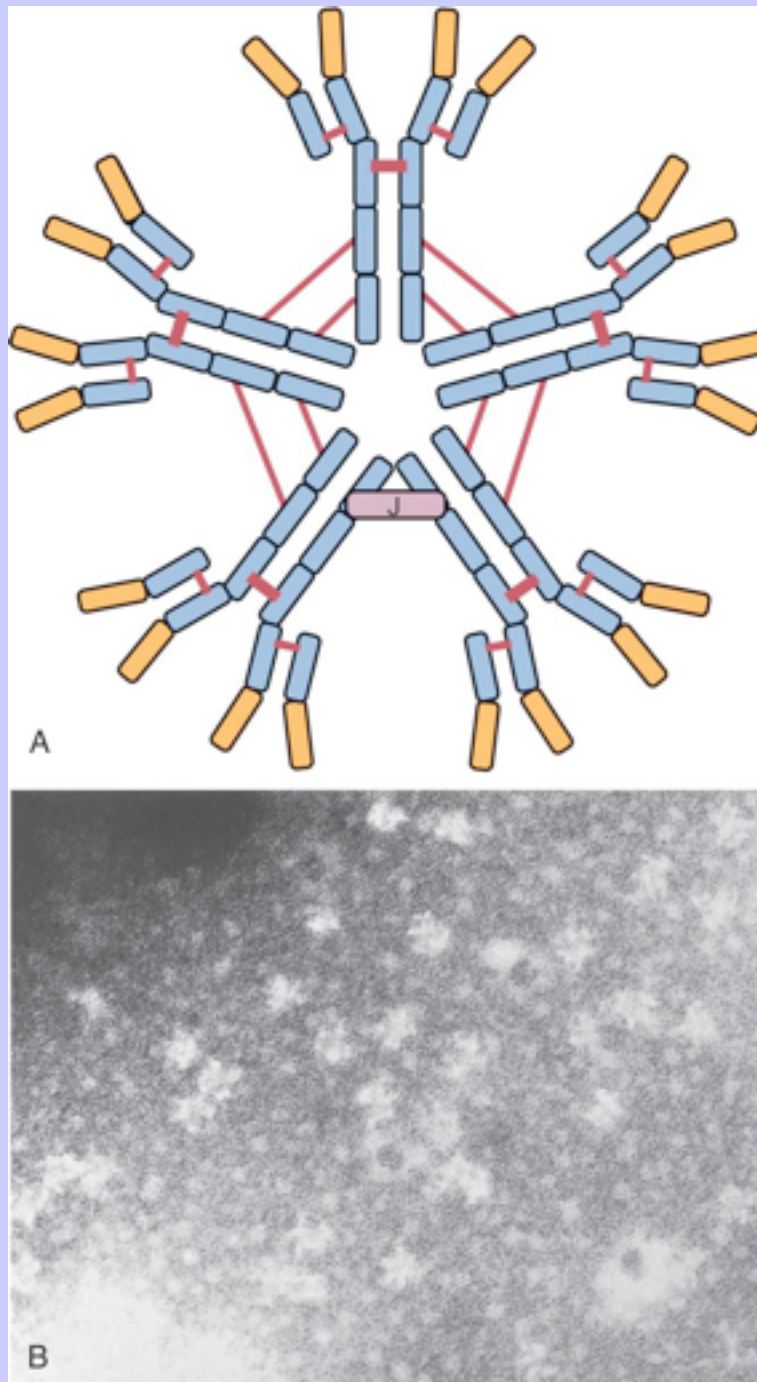
Each IgM monomer is of conventional immunoglobulin structure and so consists of two κ or λ light chains and two μ heavy chains; μ chains differ from γ chains in that they have an additional fourth constant domain (C_H4), as well as an additional 20-amino acid segment on their C terminus but have no hinge region. The complement activation site on IgM is located on the C_H4 domain.

IgM is the major immunoglobulin produced during a primary immune response (Figure 14-5). It is also produced in secondary responses, but this tends to be masked by the predominance of IgG. Although produced in small amounts, IgM is more efficient (on a molar basis) than IgG at complement activation, opsonization, neutralization of viruses, and agglutination. Because they are very large, IgM molecules rarely enter tissue fluids even at sites of acute inflammation.

14.3.3 Immunoglobulin A

IgA is secreted by plasma cells located under body surfaces. Thus it is made in the walls of the intestine,

FIGURE 14-4 The structure of IgM **(A)** and an electron micrograph **(B)** of this immunoglobulin from bovine serum ($\times 240,000$). Note that some of the molecules are five-pointed and others are six-pointed stars. (Courtesy Drs. K. Nielsen and B. Stemshorn.)



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respiratory tract, urinary system, skin, and mammary gland. Its serum concentration in most mammals is usually lower than that of IgM. IgA monomers have a molecular weight of 150 kDa, but they are normally secreted as dimers. Each IgA monomer consists of two light chains and two a heavy chains containing three constant domains. In dimeric IgA, two molecules are joined by a J chain ([Figure 14-6](#)). Higher polymers of IgA are occasionally found in serum.

IgA produced in body surfaces passes through epithelial cells into external secretions. For example, most of the IgA made in the intestinal wall is carried into the intestinal fluid. This IgA is transported through intestinal epithelial cells bound to the polymeric immunoglobulin receptor (pIgR) or secretory component. Secretory component binds IgA dimers to form a complex molecule called secretory IgA (SIgA). It protects the IgA from digestion by intestinal proteases.

Secretory IgA is the major immunoglobulin in the external secretions of nonruminants. As such, it is of critical importance in protecting the intestinal, respiratory, and urogenital tracts, the mammary gland, and the eyes against microbial invasion. IgA does not activate the classical complement pathway, nor can it act as an opsonin. It can, however, agglutinate particulate antigens and neutralize viruses. IgA prevents the adherence of invading microbes to body surfaces. Because of its importance, IgA is examined in more detail in [Chapter 19](#).

14.3.4

Immunoglobulin E

IgE, like IgA, is made by plasma cells located beneath body surfaces. It is a typical Y-shaped, four-chain immunoglobulin with four constant domains in its e heavy chains and a molecular weight of 190 kDa ([Figure 14-7](#)). IgE is, however, present in extremely low concentrations in serum. Because of this, it cannot act simply by binding and coating antigens, as the other immunoglobulins do. IgE triggers acute inflammation by acting as a signal-transducing molecule. Thus IgE molecules bind tightly to receptors (FcεRI) on mast cells and basophils. When antigen binds to this IgE, it triggers the rapid release of inflammatory molecules from the mast cells. The resulting acute inflammation enhances local defenses and helps eliminate the invader. IgE mediates Type I hypersensitivity reactions and is largely responsible for immunity to parasitic worms. IgE has the shortest half-life of all immunoglobulins (2 to 3 days) and is readily destroyed by mild heat treatment. IgE is described in more detail in [Chapter 25](#).

14.3.5

Immunoglobulin D

IgD has been found in horses, cattle, sheep, pigs, dogs, rodents, and primates but has not yet been detected in rabbits or cats. It has been identified in many different bony fish (catfish, flounder, halibut, carp, salmon, rainbow trout, fugu, zebra fish, and cod) but has not been found in chickens. IgD is a BCR mainly found attached to B cells and very little is secreted into the blood. IgD molecules consist of two δ heavy chains and two light chains but are otherwise structurally diverse. In contrast to the other immunoglobulin classes, IgD is evolutionary labile and shows many variations in structure. For example, mouse IgD lacks a Cd2 domain and thus has only two constant domains in its heavy chains. It has a molecular weight of about

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FIGURE 14-5 The relative amounts of each immunoglobulin class produced during the primary and secondary immune responses. Note that IgM predominates in a primary immune response, whereas IgG predominates in a later response.

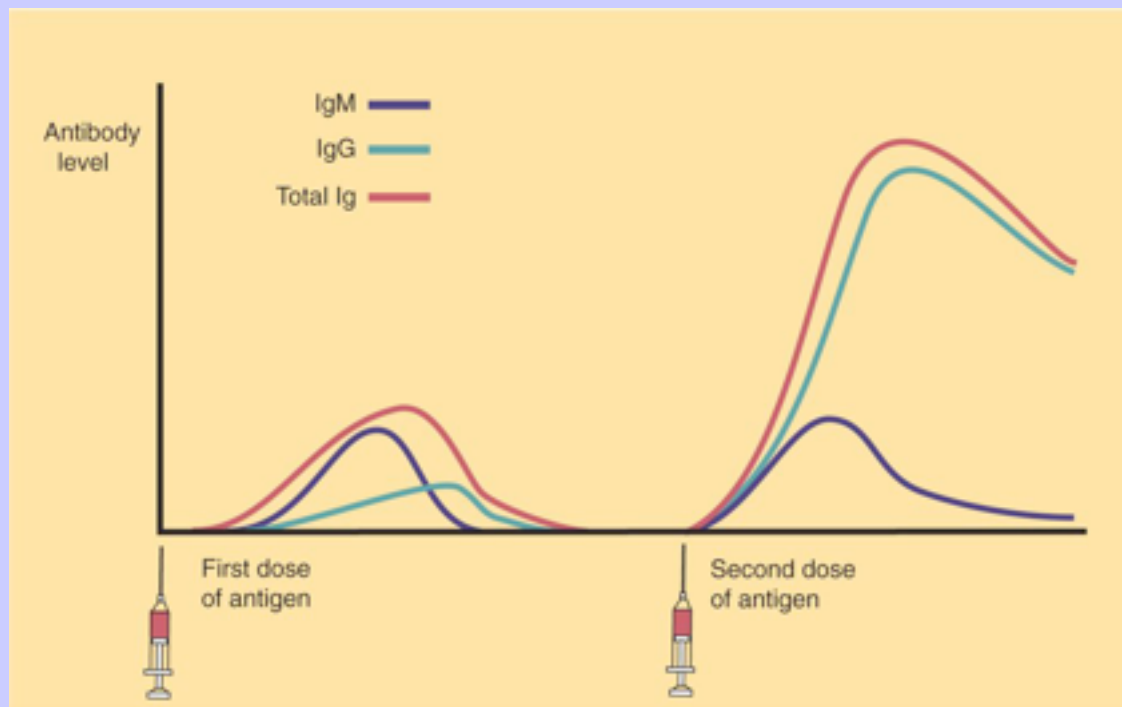


FIGURE 14-6 The structure of IgA and secretory IgA. Secretory component consists of five linked immunoglobulin domains. It is found on the surface of certain epithelial cells, where it acts as a receptor for polymeric immunoglobulins (plgR). It can also bind to IgM.

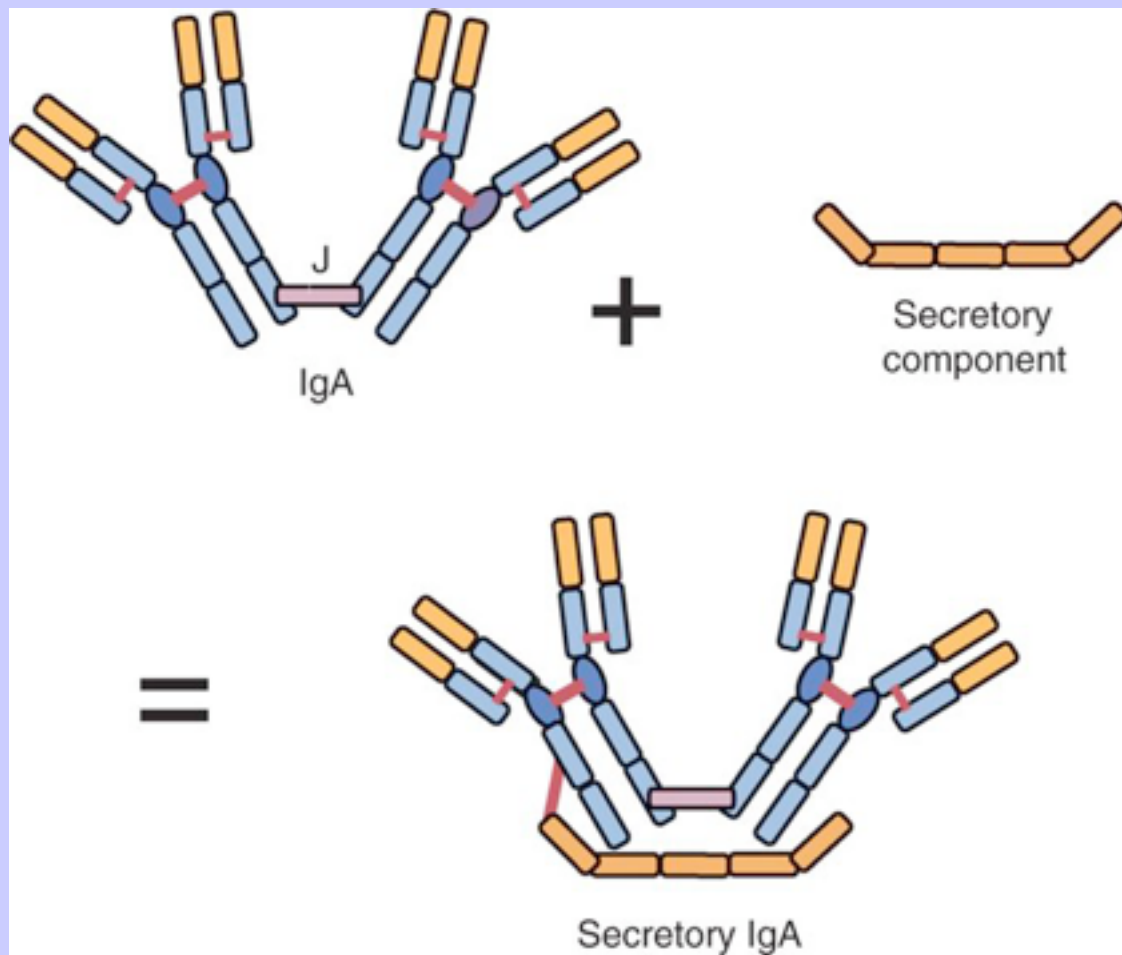
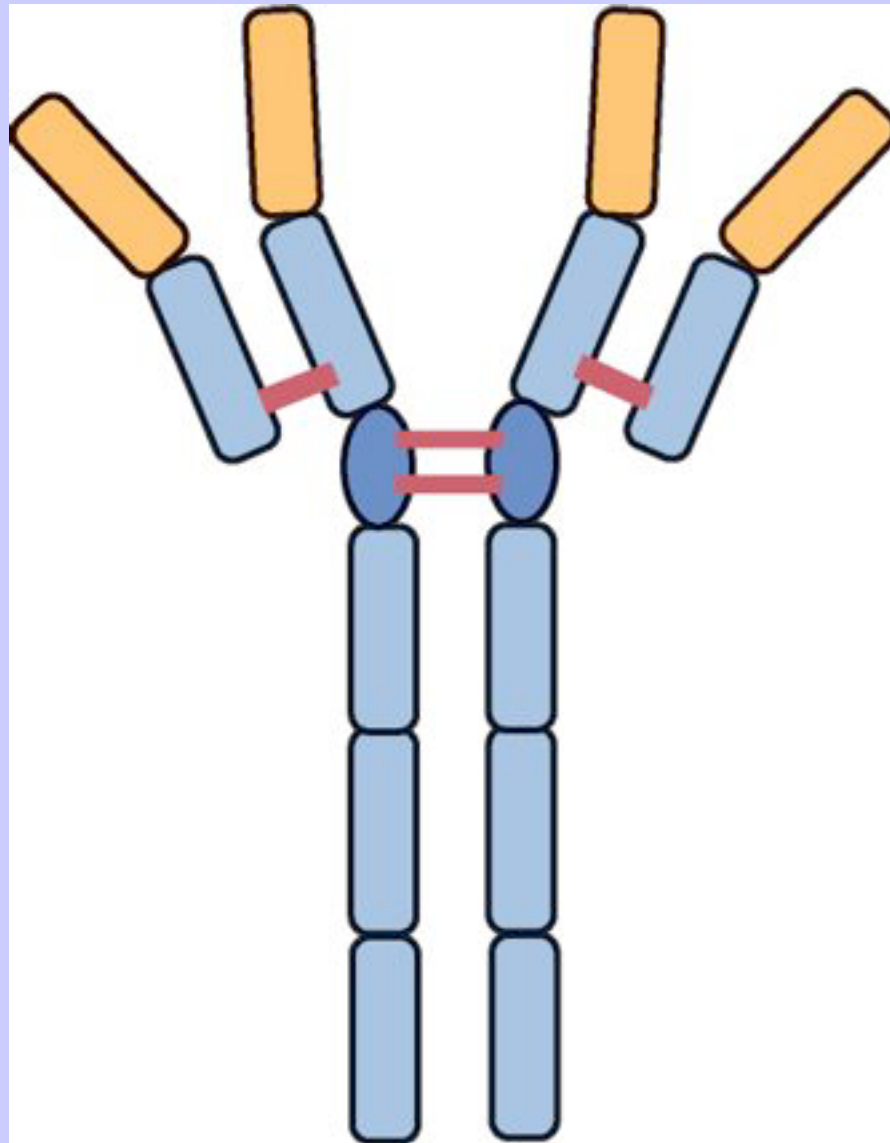


FIGURE 14-7 The structure of IgE. Note the presence of four constant domains in addition to a hinge in the heavy chain.



170 kDa ([Figure 14-8](#)). Horse, cow, sheep, dog, monkey, and human IgD, in contrast, have three heavy chain constant domains and a very long hinge domain coded for by two exons ([Figure 14-9](#)). Pig IgD has a short hinge coded for by a single exon. In cattle, sheep, and pigs, but not horses or dogs, the Cd1 domain is almost identical to the Cm1 domain of IgM, whereas the other constant domains are distinctly different. In mice, the two constant region domains (Cd1 and Cd3) are separated by a very long exposed hinge region. Because of this long hinge region and the fact that it has no interchain disulfide bonds, mouse IgD is unusually susceptible to destruction by proteases and cannot be detected in mouse serum although it may be detected in mouse plasma. Like IgE, IgD is destroyed by mild heat treatment.

14.4 THREE-DIMENSIONAL STRUCTURE OF IMMUNOGLOBULINS

Immunoglobulin peptide chains fold in a very complex manner so that an IgG molecule consists of three globular regions (two Fab regions and one Fc region) linked

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FIGURE 14-8 The structure of IgD in mice and other mammals. Note the long exposed hinge region in mouse IgD that makes this molecule very unstable.

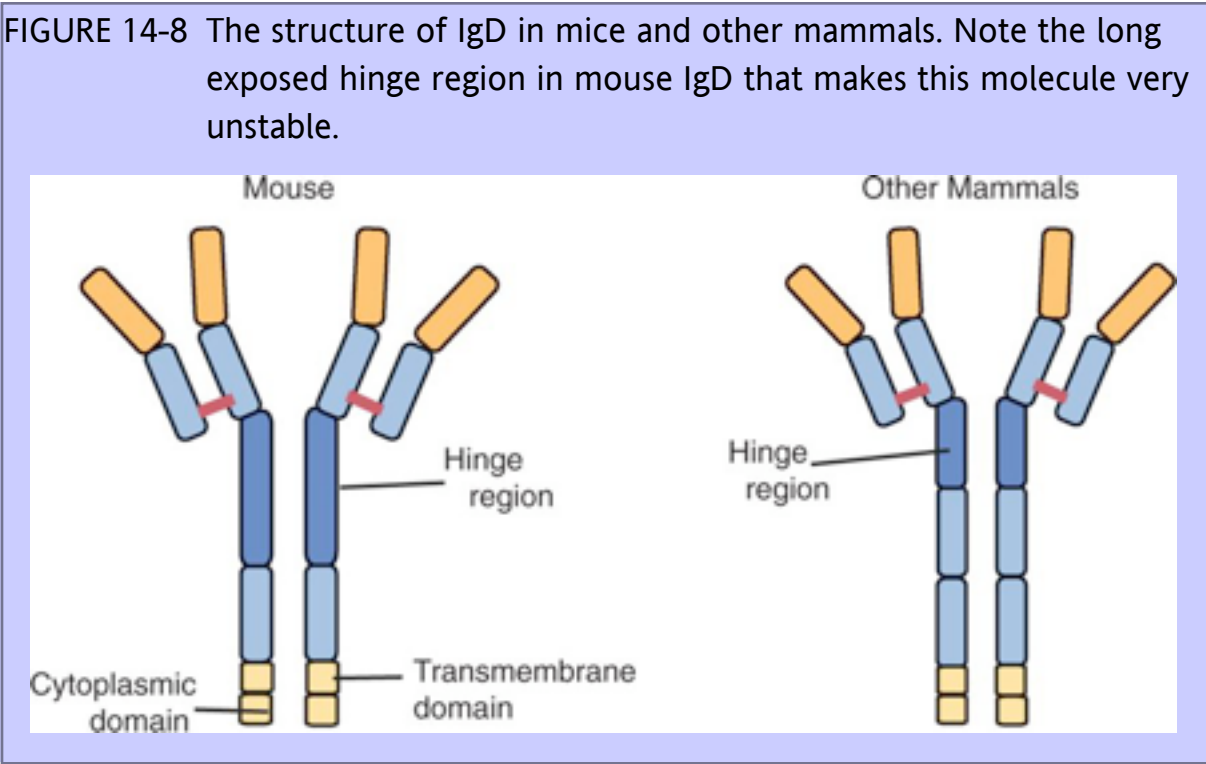
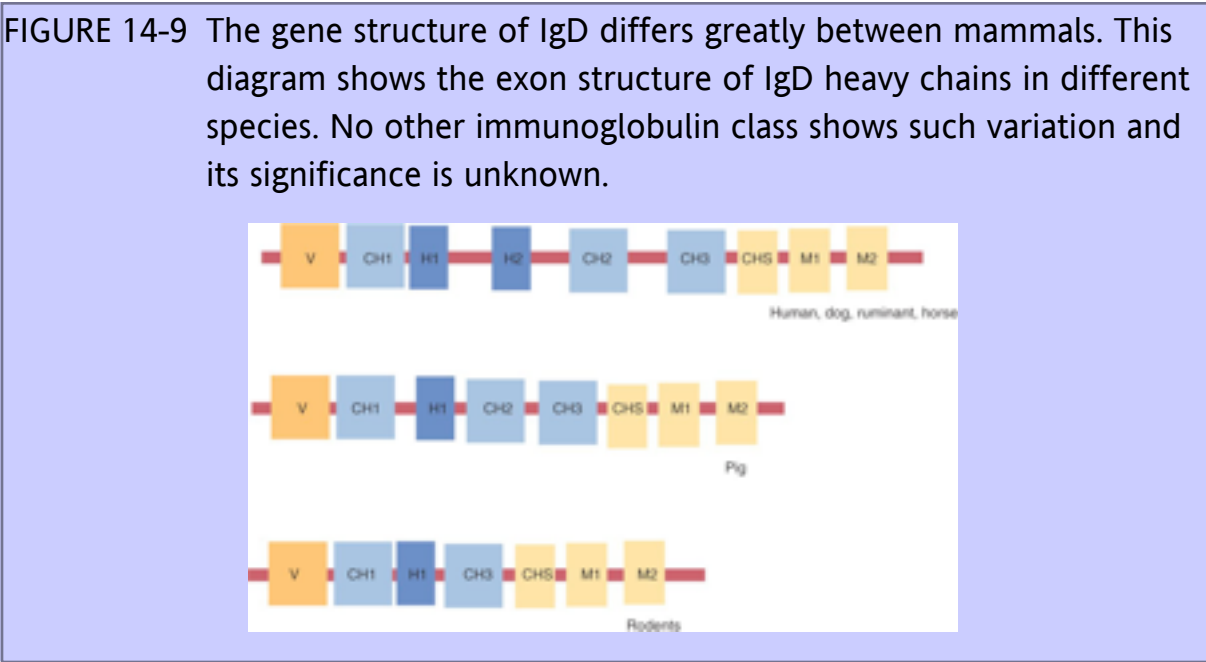


FIGURE 14-9 The gene structure of IgD differs greatly between mammals. This diagram shows the exon structure of IgD heavy chains in different species. No other immunoglobulin class shows such variation and its significance is unknown.



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by a flexible hinge ([Figure 14-10](#)). Each of these globular regions is made up of paired domains. Thus the Fab regions each consist of two interacting domains (V_H - V_L and C_{H1} - C_L), while the Fc region contains either two or three paired domains, depending on the immunoglobulin class (i.e., C_{H2} - C_{H2} , C_{H3} - C_{H3} , and in IgE or IgM, C_{H4} - C_{H4}). The peptide chains within each domain are closely intertwined. In the Fab globular regions a groove is located between the two variable domains, V_H and V_L . The amino acids of the complementarity-determining regions (CDRs) line this groove; as a result, the surface of the groove has a highly variable shape. This groove forms the antigen-binding site. The CDRs from both light and heavy chains contribute to the binding of an antigen, although the heavy chain usually contributes most to the process. Because immunoglobulins are bilaterally identical, the CDRs on each of the Fab regions are also identical. Thus the molecule has two identical antigen-binding sites and binds two identical antigens.

The presence of a hinge region in the middle of their heavy chains gives immunoglobulins such as IgG great flexibility. Since the two antigen-binding sites on each Fab region are identical, immunoglobulins are able to cross-link two antigens at the same time. Thus bacteria may be clumped together by antibody molecules in a process called agglutination. If sufficient soluble protein molecules or viruses are cross-linked by antibody, they may precipitate out of solution.

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14.5 IMMUNOGLOBULIN VARIANTS

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14.5.1 Subclasses

All immunoglobulin molecules are made of two heavy and two light chains. Several different heavy chains are employed in making these molecules. Thus when γ chains are used, the resulting immunoglobulin is IgG. IgM contains μ chains, IgA contains α chains, and so on. However, closer examination shows that even these immunoglobulin classes consist of molecules using a mixture of structurally different heavy chains known as subclasses.

Immunoglobulin subclasses have arisen as a result of gene duplication. Thus during the course of evolution, heavy chain (*IGH*) genes have been duplicated and the new gene then gradually changed through mutation. The amino acid sequences coded by these new genes may differ from the original in only minor respects. For example, bovine IgG is a mixture of three subclasses—IgG1, IgG2, and IgG3—coded for by the genes *IGHG1*, *IGHG2*, and *IGHG3*, respectively. They differ in amino acid sequence and in physical properties such as electrophoretic mobility. These immunoglobulin subclasses may also have different biological activities: for example, bovine IgG2 agglutinates antigenic particles, whereas IgG1 does not. All animals of a species will possess all these subclasses.

The number and properties of immunoglobulin subclasses vary among species. For example, most mammals have only 1 or 2 IgA subclasses, but rabbits have as many as 13. These variations among species are probably not of major biological significance; they simply reflect the number of immunoglobulin gene duplications a species has undergone.

14.5.2 Allotypes

In addition to subclass differences, individual animals show inherited variations in immunoglobulin amino acid sequences. Thus the immunoglobulins of one individual may differ from those of another individual of the same species ([Figure 14-11](#)). These inherited sequence variations in heavy chain genes are called allotypes.

14.5.3 Idiotypes

The third group of structural variants found in immunoglobulins results from the variations in the amino acid sequences within the variable domains on light

FIGURE 14-10 A computer-generated molecular model of IgG. It is instructive to compare this with the diagrams of IgG structure seen earlier in this chapter. (Courtesy Dr. S. Linthicum.)

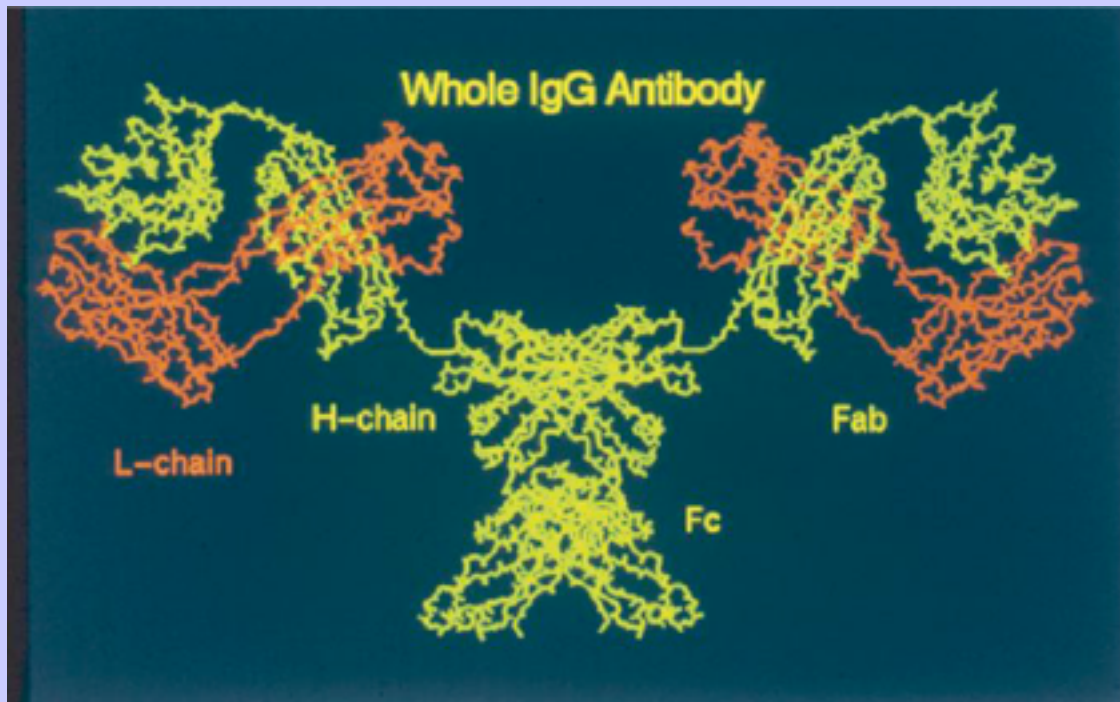
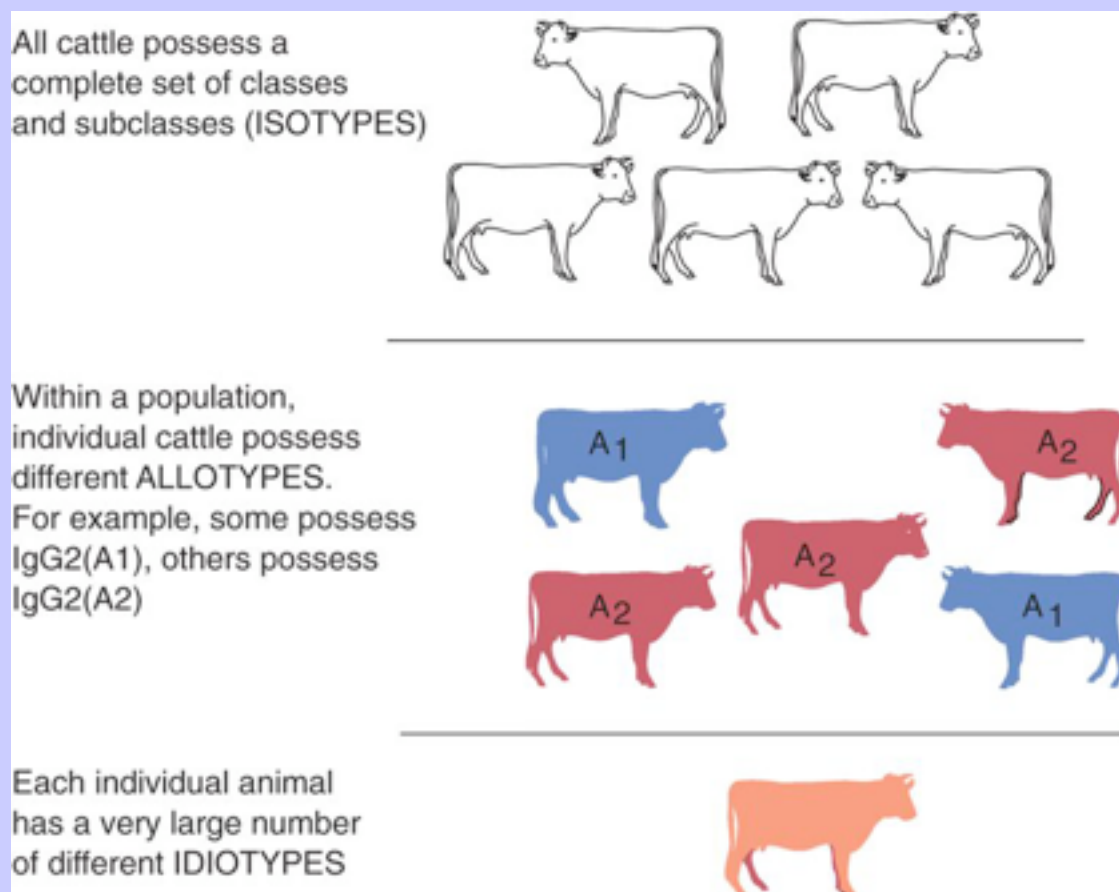


FIGURE 14-11 A schematic diagram showing the differences among the inheritance of the major immunoglobulin variants.



and heavy chains. These variants are called idiotopes. The collection of idiotopes on an immunoglobulin is called its idio-
type. Some idiotopes may be located within the antigen-binding site. Others are located on nonantigen
binding areas of the V domain.

14.6 PRODUCTION OF IMMUNOGLOBULIN HEAVY CHAINS

Two different genes code for each immunoglobulin heavy chain. One gene codes for the variable domain (and thus the antigen-binding site), whereas a separate gene codes for the constant domains. The way in which genes can code for the variable domains is discussed in [Chapter 15](#). The genes that code for the constant region of immunoglobulin heavy chain (*IGH* genes) each consist of several exons (expressed sequences). Each exon codes for a constant domain, and one codes for the hinge region ([Figure 14-12](#)). A complete IgM constant region gene (*IGHM*) therefore consists of five exons, whereas an IgA constant region gene (*IGHA*) contains four exons. All the heavy chain constant region genes are located together on one chromosome. They are generally arranged in the order 5'-*IGHM-IGHD-IGHG-IGHE-IGHA*-3'. Thus all the genes for μ chains are followed by the genes for δ chains, which are in turn followed by the γ chain genes and so on.

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During their life span, B cells undergo two different DNA recombination events. The first, called V(D)J recombination, creates the antigen binding site of the B cells as they develop within the bone marrow in the absence of antigens. Later in life, when antigens activate the B cells, a second phase of DNA recombination occurs. This second phase changes the class of antibody produced by a B cell. This class switch recombination does not affect the antigen-binding specificity of a cell but results in the production of a different heavy chain constant region.

14.6.1 Class Switch Recombination

During the course of an antibody response, immunoglobulin classes change, although their antigen-binding ability does not. This “class switch” can be explained by the way in which heavy chain genes are constructed and used.

During an antibody response, the immunoglobulin classes are synthesized in a standard sequence. Thus a B cell first uses the *IGHM* genes to make IgM BCRs. The remaining genes located 3' to *IGHM* are ignored. In species that make IgD, the B cell also transcribes the *IGHD* genes and then expresses both IgM and IgD. Eventually, however, as the immune response progresses, a responding B cell switches to using *IGHG*, *IGHA*, or *IGHE* genes and becomes committed to synthesizing BCRs and immunoglobulins of one of the other major classes—namely, IgG, IgA, or IgE. The unwanted, unused IGH genes are excised as a DNA circle and lost from the cell while the required IGH gene is spliced directly to the IGHV genes.

For example, if IgM is to be synthesized, the IGHV genes are spliced directly to the IGHM genes ([Figure 14-13](#)). On the other hand, if IgA is to be synthesized, the genes coding for C_μ to C_ε inclusive are deleted and the IGHV genes are then spliced directly to the IGHA genes. There are several ways by which these

FIGURE 14-12 A peptide chain such as an immunoglobulin heavy chain is coded for by a series of exons separated by intervening sequences or introns. Usually each exon codes for a single domain.

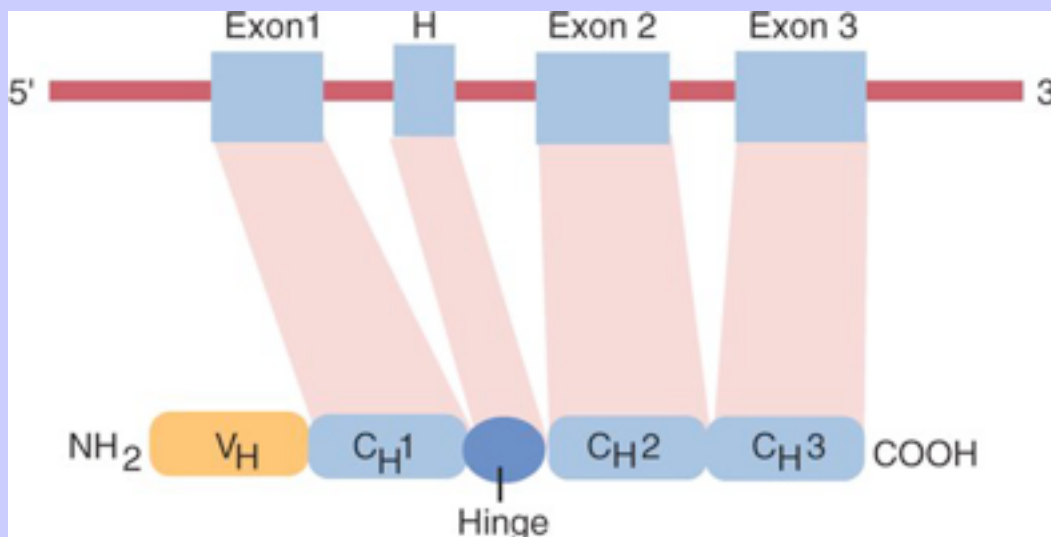
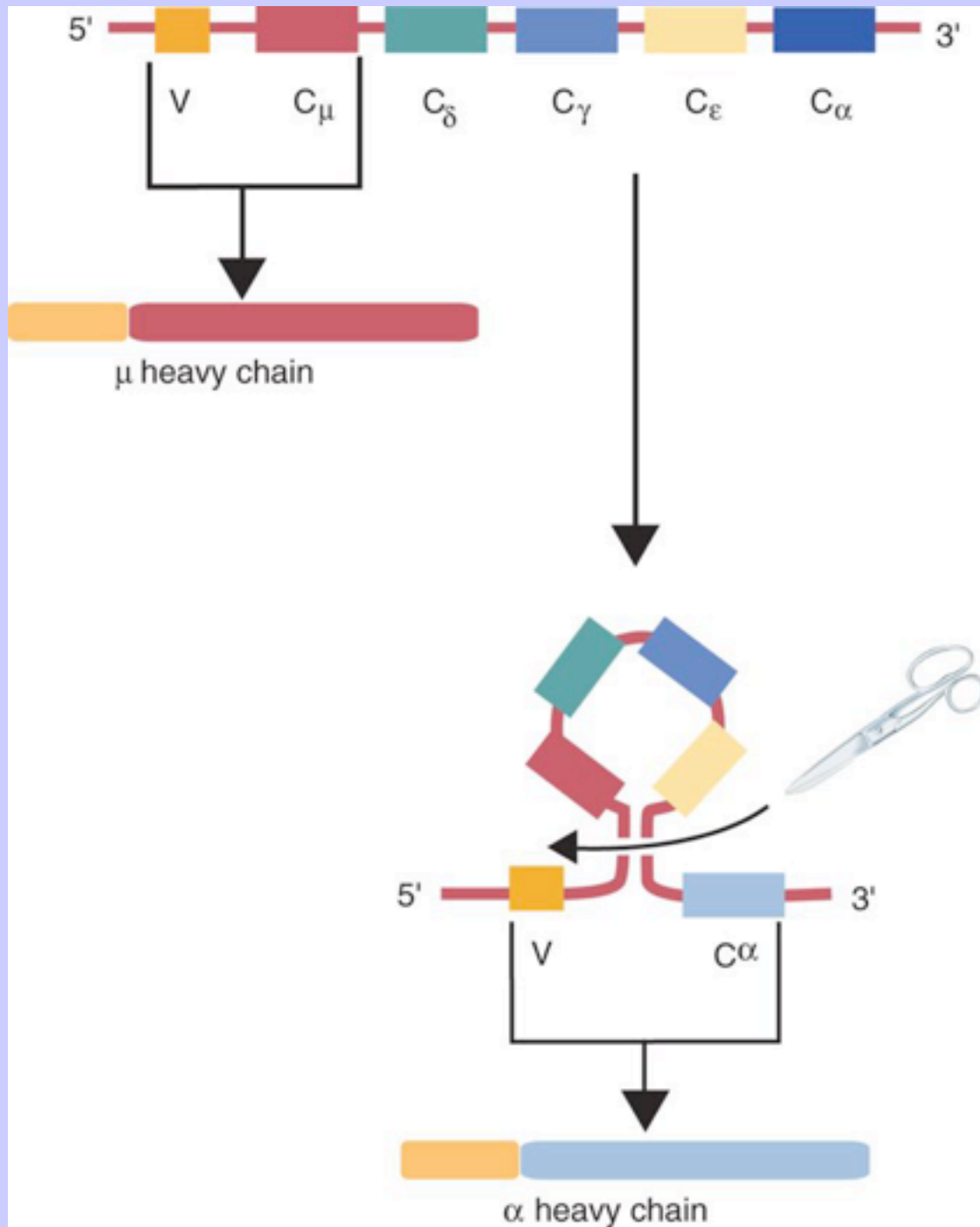


FIGURE 14-13 The mechanism of class switching. In this example a switch is made from IgM production to IgA production.



intervening genes can be excised. The simplest is called looping out-deletion. In this case the V region and C gene segments come together by looping out and then excising the intervening DNA using an enzyme called a

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recombinase. Two signals are needed to initiate class switching in a B cell. First, the B cell must receive an activation signal. This comes from cross-linking between CD40 on the B cell and CD154 on a helper T cell. Second, the specific class switch must be determined. This choice is regulated by cytokines, especially by interleukin-4, transforming growth factor- β , and interferon- γ . Signals from the CD40 and antigen activate the recombinase in the B cell while the cytokines, by activating specific promoter regions, target the recombinase to a specific immunoglobulin gene.

14.6.2 BCRs and Soluble Immunoglobulins

Immunoglobulins can exist either as BCRs or as secreted antibodies. The heavy chain of a BCR contains a hydrophobic transmembrane C-terminal domain that attaches it to a B cell. This domain is absent from the secreted antibody. The switch between the two forms depends on the differential splicing of exons. For example, in the *IGHM* gene, there are two short exons, $C\mu S$ and $C\mu M$, located 3' to $C\mu 4$ ([Figure 14-14](#)). $C\mu S$ codes for the C-terminal domain of the secreted form, whereas $C\mu M$ codes for the hydrophobic domain of the cell-bound form. When IgM is made, all the $C\mu$ exons are first transcribed to mRNA. To produce cell-bound IgM, the mRNA is cleaved so that the $C\mu S$ exon is deleted and the $C\mu 4$ exon is spliced directly to the $C\mu M$ exon. To produce secreted IgM, the exon coding for the $C\mu M$ domain is deleted and translation is stopped after $C\mu 4$ and $C\mu S$ are read.

14.7 IMMUNOGLOBULINS OF DOMESTIC MAMMALS

All mammals possess genes for and express four or five major immunoglobulin classes (IgG, IgM, IgA, IgE, and IgD), although these may not have been formally identified in all species ([Table 14-3](#)). The basic characteristics of each of these classes are as described previously. However, during the course of evolution, as pointed out above, the immunoglobulin heavy chain (IGH) genes have duplicated, sometimes several times. These duplicated genes can then mutate so that mammals may produce several different subclasses of a specific immunoglobulin. If a duplicated gene mutates in such a way that it is no longer functional, it becomes a pseudogene. The number of duplications and hence the number of immunoglobulin subclasses and pseudogenes varies greatly among species. In looking at these species differences, the reader might gain additional insight by examining the phylogeny of domestic animal species (see [Figures 37-13](#) and [37-14](#)).

14.7.1 Horses

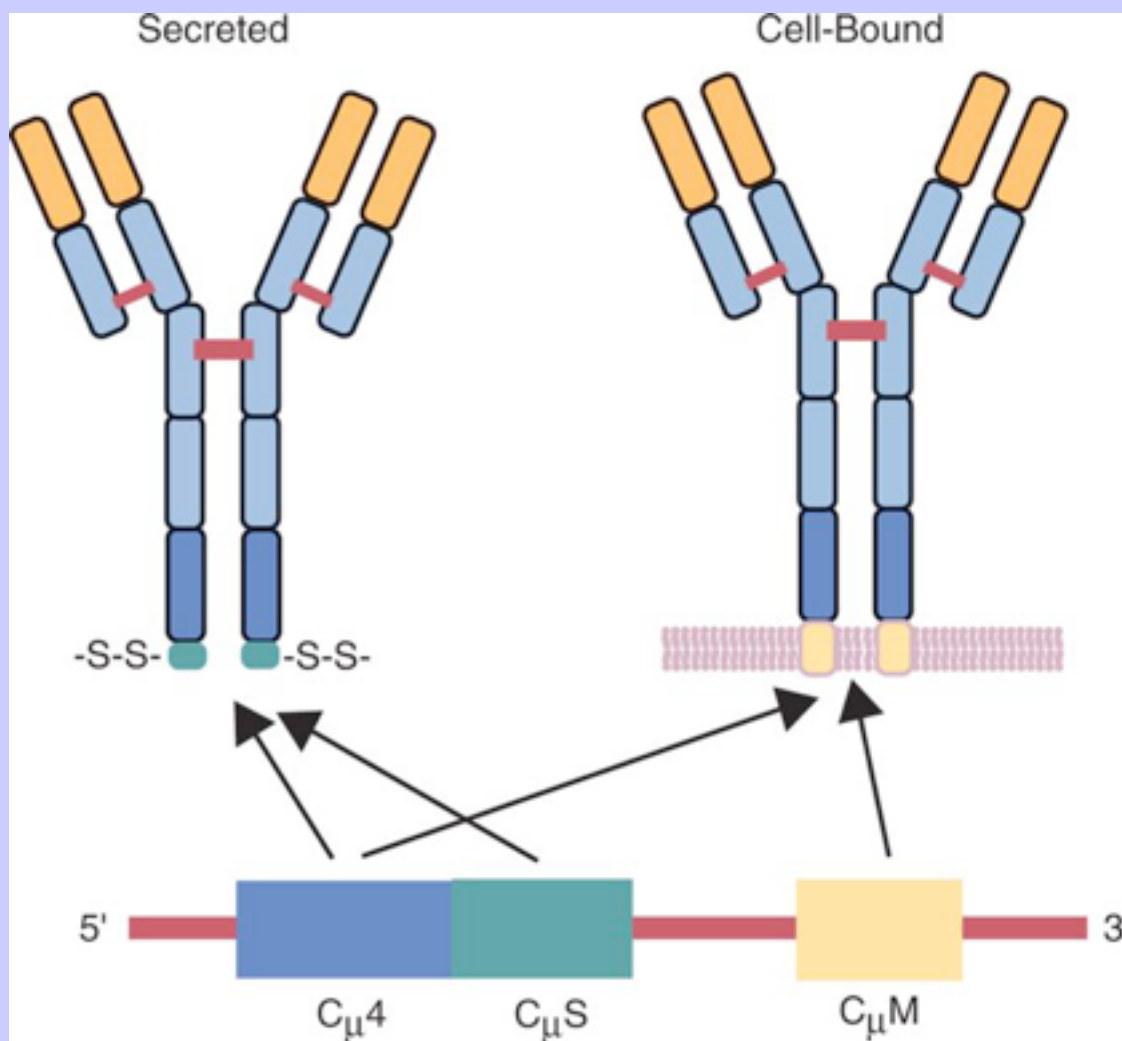
The horse has seven IGHG genes, and all are expressed. Thus there are seven IgG subclasses: IgG1 (IgGa), IgG2 (IgGc), IgG3 (IgG[T]), IgG4 (IgGb), IgG5, IgG6 (IgG[B]), and IgG7. (The previously used designation for IgG3—IgG[T]—was originally derived from the observation that this subclass predominates in the serum of horses used for tetanus immune globulin production.) IgG3 does not activate guinea pig complement and reacts in a precipitation reaction by a rather characteristic flocculation. The order of the Ig heavy chain genes in the horse is now: 5'-M-D-G1-G2-G3-G7-G4-G6-G5-E-A-3'. The gene, coding for IgG7, is closely related to IGHG4 and likely emerged from a recent duplication of the IGHG4 gene. The horse heavy chain gene locus is located on chromosome 24qtr. This corresponds to human chromosome 14, where the human IGH locus is located. Horses also possess and express IgM, IgD, IgA, and IgE. The horse IGHD gene is located downstream from IGHM. It appears to be expressed at least at the mRNA level. Horses have two IgG4 allotypes (IgG4^a and IgG4^b) and four IgE allotypes (IgE¹⁻⁴).

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14.7.2 Cattle

Cattle have three *IGHG* genes and thus three subclasses: IgG1, IgG2, and IgG3. IgG1 constitutes about

FIGURE 14-14 Immunoglobulins serving as B cell antigen receptors have a hydrophobic transmembrane C-terminus. In contrast, the secreted form lacks this sequence. The difference between the two forms is determined by RNA splicing following transcription.



50% of the serum IgG and is remarkable for being the predominant immunoglobulin in cows' milk rather than IgA. IgG2 levels are highly heritable; thus concentrations vary greatly among cattle. Cattle possess a unique Fc receptor on their macrophages and neutrophils that is structurally unlike any other Fc receptor and binds only IgG2. Since bovine IgG2 has a very small hinge region, this receptor might represent a special adaptation to the

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structure of this immunoglobulin. Two heavy chain allotypes (a and b) have been identified in all three classes. Allotype B1 is found on light chains of some cattle but is relatively uncommon. IgA, IgM, and IgE also occur in cattle. Cattle have functional *IGHD* genes, and IgD may be expressed on the B cell surface. Cattle are also unique in that they have two *IGHM* genes, although one, the *IGHML*-gene, is a pseudogene located on chromosome 9. The functional *IGHM* gene is located on chromosome 21 together with the other heavy chain genes.

Table 14-3 Immunoglobulin Classes and Subclasses in Selected Mammals

Species	Immunoglobulin Classes				
	IgG	IgA	IgM	IgE	IgD
Horse	G1, G2, G3, G4, G5, G6, G7	A	M	E	D
Cattle	G1, G2, G3	A	M	E	D
Sheep	G1, G2, G3	A1, A2	M	E	D
Pig	G1, G2a, G2b, G3, G4	A	M	E	D
Dog	G1, G2, G3, G4	A	M	E1, E2	D
Cat	G1, G2, G3, (G4?)	A	M	(E1, E2?)	?
Mouse	G1, G2a, G2b, G3	A1, A2	M	E	D
Chimpanzee	G1, G2, G3,	A	M	E	D
Human	G1, G2, G3, G4	A1, A2	M1, M2	E	D

14.7.2.1 Box 14-1 The Curious Case of the Camel

Members of the camel family from both the old and new worlds (camels and llamas) have three IgG subclasses: IgG1, IgG2, and IgG3. IgG1 has a conventional four-chain structure and therefore has a molecular weight of 170 kDa. In contrast, IgG2 and IgG3, which together account for 75% of camel immunoglobulins, are 100-kDa heavy chain dimers that have no light chains. In addition, camel IgG2 heavy chains lack a CH1 domain but compensate for this by having a very long hinge region. Despite lacking light chains, these molecules can still bind to many antigens. It has been noted that these antibodies appear to bind to the substrate pockets of enzymes. Studies have now shown that the antigen-binding site on these heavy chains (the paratope) is very convex. This enables it to fit snugly into the concave active site on an enzyme. Thus these single chain antibodies may have a structural advantage over conventional immunoglobulins in neutralizing enzyme activity.

14.7.3 Sheep

The immunoglobulin subclasses of sheep are similar to those of cattle, with three *IGHG* genes coding for IgG1, IgG2, and IgG3. Some sheep have an IgG1a allotype. An *IGHD* gene has been detected in sheep. Three IgA heavy chain allotypes have been identified.

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14.7.4 Pigs

Pigs have at least five IgG subclasses—named IgG1, IgG2a, IgG2b, IgG3, and IgG4. Whether all deserve to be called subclasses is unclear. For example, IgG2a and IgG2b differ by only three amino acids. However, DNA analysis has indicated that pigs may have 8 to 12 distinct *IGHG* genes. Presumably the unused genes are pseudogenes. IgG is the predominant serum immunoglobulin accounting for about 85% of the total. IgM accounts for about 12%, and dimeric IgA for about 3% of serum immunoglobulins. Pigs have a single *IGHA* gene that occurs in two codominant allelic variants. One form, *IGHAa*, has a normal hinge region with six amino acids. The other allele, *IGHAb*, has a deletion mutation so that its hinge contains only two amino acids. The biological consequences of this are unclear. The first heavy chain constant domain of pig IgD may be coded by either a CH1 δ gene or by a CH1 μ gene. Thus pig IgD heavy chains can be coded by “VDJ-CH1 μ -CH2 δ -CH3 δ ,” or by “VDJ-CH1 δ -CH2 δ -CH3 δ .” This pattern has not been reported in other mammals. These two genes do however show 98.7% similarity, so the biological consequences are probably not great. Swine IgE has also been identified. Four IgG allotypes and one IgM allotype have been reported ([Box 14-1](#)).

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14.7.5 Dogs and Cats

Dogs have four *IGHG* genes and hence four IgG subclasses, named IgG1, IgG2, IgG3, and IgG4 in order of abundance. (These were previously called IgG-A, -B, -C, and -D). In addition, dogs have IgA, IgM, IgD, and IgE. Preliminary evidence also suggests that they may have two IgE subclasses, IgE1 and IgE2. Four allelic variants have been identified in the dog *IGHA* gene. All are restricted to the hinge region.

Cats have at least three, and possibly four, *IGHG* genes (IgG1, IgG2, IgG3, and IgG4), one IgM subclass, and possibly two IgA subclasses (IgA1 and IgA2), as well as two possible IgE subclasses. An IgM allotype has been described in the dog.

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14.7.6 Primates

Humans have four *IGHG* genes coding for IgG1 to IgG4. Chimpanzees and rhesus macaques possess three *IGHG* genes coding for IgG1, IgG2, and IgG3. The chimpanzee IgG2 molecule contains epitopes also found on both human IgG2 and IgG4, suggesting that the *IGHG2* and *IGHG4* genes split after humans separated from chimpanzees. Baboons (*Papio cynocephalus*) have four *IGHG* genes, but they differ significantly from human IgG in their hinge region. Rhesus macaques may have two IgM subclasses. All the great apes, with the exception of the orangutan, have two IgA subclasses.

14.7.7 Other Mammals

Rats and mice have four or five functional *IGHG* genes. In contrast, rabbits have only one *IGHG* gene despite having 13 *IGHA* genes, at least 12 of which are functional. They appear to lack IgD. The expression of these IgA subclasses varies among different tissues

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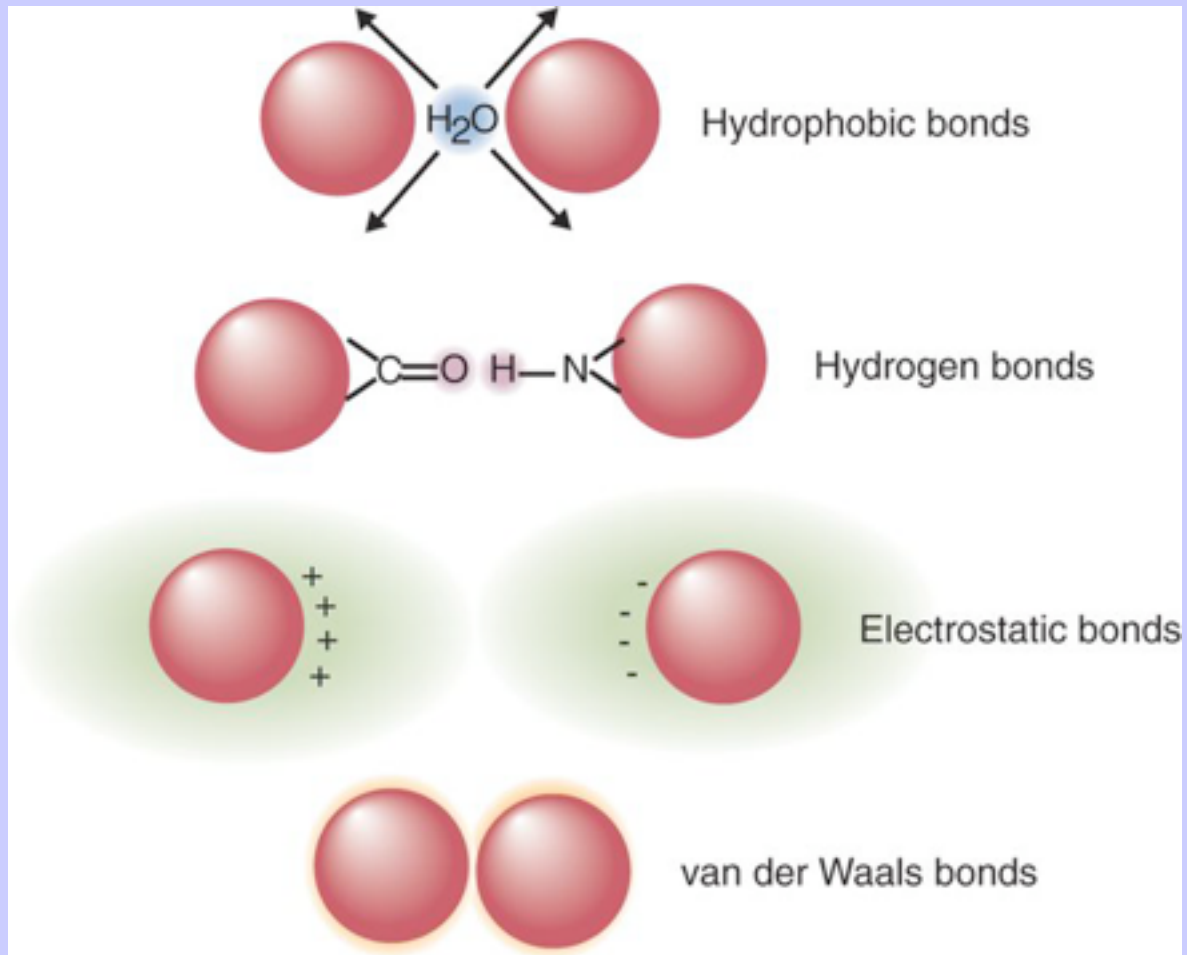
¹⁵ CHAPTER 15 How Antigen-Binding Receptors Are Made

^{15.1} KEY POINTS

- Antigen receptors must reliably bind foreign antigens on their first encounter. This is achieved by scrambling and rearranging the peptide sequences that comprise the antigen-binding site, which generates millions of different structural conformations. These newly generated receptors can bind to almost any invading microorganism.
- Antigen binds to a T cell antigen receptor (TCR) or B cell antigen receptor (BCR) when its shape matches the conformation of the groove in the antigen-binding receptor.
- The shape of the antigen-binding groove depends on the sequence of the amino acids that line the groove. The sequence of the amino acids depends on the nucleotide sequence in the genes encoding the receptor.
- Because of the way nucleotide sequences can be rearranged in these genes, an enormous number of different BCRs and TCRs can be generated.
- In some mammals, variable regions may be constructed by means of gene segment recombination. Different gene segments selected at random from a large library are joined to generate great diversity.
- In other mammals, receptor diversity is generated by gene conversion. Small blocks of donor nucleotides are inserted into V region genes to generate diversity.
- Antigen-binding sites in BCRs, but not TCRs, also undergo somatic mutation.
- All these mechanisms collectively enable an animal to make the millions of different receptors that can bind to almost all foreign antigens.

One of the central problems encountered in understanding acquired immunity is how lymphocytes recognize the enormous diversity of microbes that may invade the body. Given that microorganisms change rapidly, the immune system must be able to respond not only to existing organisms but also, within reason, to newly evolved organisms. This

FIGURE 15-1 Noncovalent bonds that link an antigen with its receptor arranged in order of relative importance. All these bonds are effective only over a very short distance. It is therefore essential that the shape of the antigen and its receptor site match very well if strong binding is to be achieved.



implies that any animal must be able to produce a huge number of different B cell antigen receptors. Similar considerations apply to T cell antigen receptors. The ability of the acquired immune responses to respond specifically to an enormous number of foreign antigens implies the existence of an enormous number of different lymphocytes, each with its own specific antigen receptors. The antigen receptors on T and B cells have not evolved to deal with specific microbial antigens, but the repertoire of T cell antigen receptors (TCRs) and B cell antigen receptors (BCRs) is so enormous that at least some of these receptors will bind to any individual antigen.

The ability of a receptor to bind to an antigen is determined by the shape of its binding site. This shape depends on the folding of its peptide chains, which is governed in turn by their amino acid sequences. Each amino acid in a peptide chain exerts an influence on its neighboring amino acids that determines their relative orientation. The shape of a peptide chain therefore represents the contributions of all amino acids in the chain as the peptide assumes its most

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energetically favorable conformation. The shape of a protein is determined by its amino acid sequence, and that sequence is determined by the sequence of bases in the DNA coding for that protein.

15.2 RECEPTOR-ANTIGEN BINDING

When an antigen and its receptor combine, they interact through the chemical groups on the surface of the antigen and on the complementarity-determining regions (CDRs) of the receptor. In classical chemical reactions, molecules are assembled through the establishment of firm covalent bonds. These bonds can be broken only by the input of a large amount of energy—energy that is not readily available in the body. In contrast, the formation of noncovalent bonds provides a rapid and reversible way of forming complexes and permits reuse of molecules in a way that covalent bonding would not allow. However, noncovalent bonds act over short intermolecular distances and, as a result, form only when two molecules approach each other very closely. The binding of an antigen to a BCR or TCR is exclusively noncovalent, so the strongest binding occurs when the shape of the antigen and the shape of the receptor conform to each other. This requirement for a close conformational fit has been likened to the specificity of a key for its lock.

The major bonds formed between an antigen and its receptor are hydrophobic ([Figure 15-1](#)). When antigen and antibody molecules come together, they exclude water molecules from the area of contact. This exclusion frees some water molecules from constraints imposed by the proteins and is therefore energetically stable. (The bond can be likened to two wet glass microscope slides stuck together. Anyone who has tried to separate wet slides can confirm the effectiveness of this type of bonding.)

A second type of binding between an antigen and its receptor is mediated by hydrogen bonds. When a hydrogen atom covalently bound to one electronegative atom (e.g., an —OH group) approaches another electronegative atom

(e.g., an $\text{O}=\text{C}-$ group), the hydrogen is shared between the two electronegative atoms. This situation is energetically favorable and is called a hydrogen bond. The major hydrogen bonds formed in antigen-receptor interaction are $\text{O}-\text{H}-\text{O}$, $\text{N}-\text{H}-\text{N}$, and $\text{O}-\text{H}-\text{N}$. Hydrogen bonds are normally present between proteins and water molecules in aqueous solution, so the binding of an antigen to its receptor by hydrogen bonds requires relatively little net energy change.

Electrostatic bonds formed between oppositely charged amino acids may contribute to antigen-receptor binding, but the charge on many protein groups is commonly neutralized by electrolytes in solution. As a result, the relative importance of electrostatic bonds is unclear.

When two atoms approach very closely, a nonspecific attractive force, called a van der Waals force, becomes operative. It occurs as a result of a minor asymmetry in the charge of an atom because of the position of its electrons. This force, though very weak, may become collectively important when two large molecules come into contact. It can therefore contribute to antigen-receptor binding.

The binding of a receptor to its antigen is therefore mediated by multiple noncovalent bonds. Each bond is relatively weak in itself, but collectively the bonds may have a significant binding strength. All these bonds act only across short distances and weaken rapidly as that distance increases. Electrostatic bond and hydrogen bond strengths are inversely proportional to the square of the distance between the interacting molecules; the van der Waals forces and hydrophobic forces are inversely proportional to the seventh power of that distance. Thus the strongest binding between an antigen and its receptors occurs when their shapes match perfectly and multiple noncovalent bonds form. Antigens can bind to receptors when they fit less than perfectly, but the strength of binding will be reduced.

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15.3 ANTIGEN RECEPTOR GENES

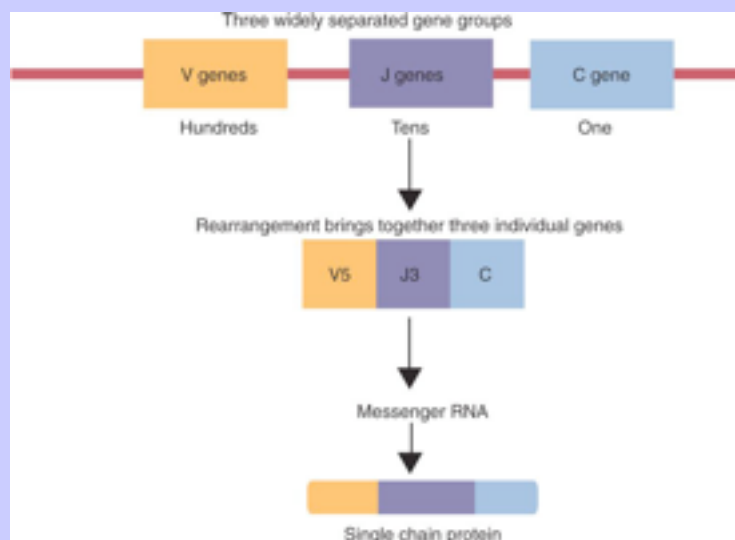
The information needed to make all proteins, including antigen receptors, is stored in an animal's genome. Thus all that is required for the production of these molecules is that the necessary genes be turned on. Once the appropriate genes are activated, they can be transcribed into RNA and translated into the appropriate receptor protein on B or T cells. It has been estimated that mammals can produce up to 10^{15} different antigen receptors to be expressed on B and T cells but that in order to produce this enormous diversity they use fewer than 500 genes.

The key to generating this enormous receptor diversity lies in that fact that multiple genes code for each receptor peptide chain. Several genes code for each variable region whereas only one codes for a constant region. As a result, the single constant-region gene can be combined with any one of several different variable-region genes to make a complete receptor peptide chain ([Figure 15-2](#)). Instead of having to store genes for all possible receptor chains, it is only necessary to store the genes for all the variable regions and to match these to an appropriate constant-region gene when needed. In addition, antigen receptor chains may be paired in different combinations to yield even greater diversity, a process called combinatorial association.

15.4 IMMUNOGLOBULIN/B CELL RECEPTOR DIVERSITY

In order to make as many different antibodies as possible, it is necessary to diversify the amino acid sequences of the variable domains in both light and heavy chains. Since these amino acid sequences are determined by the nucleotide sequences in the genes coding for these variable regions, mechanisms must exist for generating this nucleotide sequence diversity. In practice, this nucleotide sequence diversity is mediated through three distinct mechanisms: gene recombination, somatic mutation, and gene conversion. All three mechanisms alter and diversify

FIGURE 15-2 Antigen receptor chains are coded for by three genes originating in three widely separated groups of genes. The genes for a complete receptor chain are assembled by joining one gene selected from each group.



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antibody genes in such a way that an incredibly diverse array of antigen receptors is generated. The relative importance of each of these mechanisms differs between species so that the diversity mechanisms that operate in humans and mice are not the same as those that operate in domestic mammals.

15.5 GENE RECOMBINATION

Gene recombination results from the random selection of one gene from each of several groups of genes followed by recombination of the selected genes to generate sequence diversity. It is well seen in the genes that code for immunoglobulins.

Three gene clusters code for immunoglobulin peptide chains and each is found on a different chromosome ([Figure 15-3](#)). One cluster, called *IGK*, codes for κ light chains; one, called *IGL*, codes for λ light chains; and one, called *IGH*, codes for heavy chains.

15.5.1 The IGL Cluster

Each λ light chain is coded for by three genes from the *IGL* cluster. These are called *IGLV*, *IGLJ*, and *IGLC*. The *IGLV* gene codes for most of the variable region up to position 95 from the N terminus. The *IGLC* gene codes for the constant region starting at position 110. The intervening 15 amino acids are coded by a short gene called *IGLJ*. In humans each *IGL* cluster contains about 100 different *IGLV*, six *IGLJ*, and three *IGLC* genes. (The three *IGLC* genes code for three λ chain subtypes.) The *IGL* cluster in cattle contains about 20 *IGLV* genes, but 14 of these are pseudogenes. Many of the pseudogenes are fused to *IGLJ* in the germ line, suggesting that they are probably not expressed. There is more than one *IGLJ* gene but only one is expressed. There are 90 to 100 *IGLV* genes in sheep but only one *IGLJ* gene.

15.5.2 The IGK Cluster

κ light chains are also coded for by three genes, *IGKV*, *IGKJ*, and *IGKC*. In the human *IGK* cluster, for example, there are 40 different *IGKV* genes, five different *IGKJ* genes, and a single *IGLC* gene. Horse *IGK* contains up to 30 *IGKV* genes, three *IGKJ* genes, and a single *IGKC* gene.

15.5.3 The IGH Cluster

In humans, heavy chain V regions are coded for by three genes, *IGHV*, *IGHD*, and *IGHJ*. The *IGH* cluster contains about 90 different *IGHV* genes. Mouse *IGH* may have as many as 1500 different *IGHV* genes, but up to 40% of these are pseudogenes. The *IGH* cluster also contains several *IGHJ* genes situated 3' to the *IGHV* genes. Several short genes, called *IGHD* genes (D for diversity), are located between the *IGHV* and *IGHJ* genes (see [Figure 15-3](#)). In mice there are about 12 *IGHD* genes, and in humans there are at least 30.

A large noncoding region separates the *IGHJ* genes from the *IGHC* genes. The *IGHC* genes consist of a series of constant-region genes, one for each heavy chain class and subclass, arranged in the order 5'-C μ -C δ -C γ -C ϵ -C α -3' along the chromosome.

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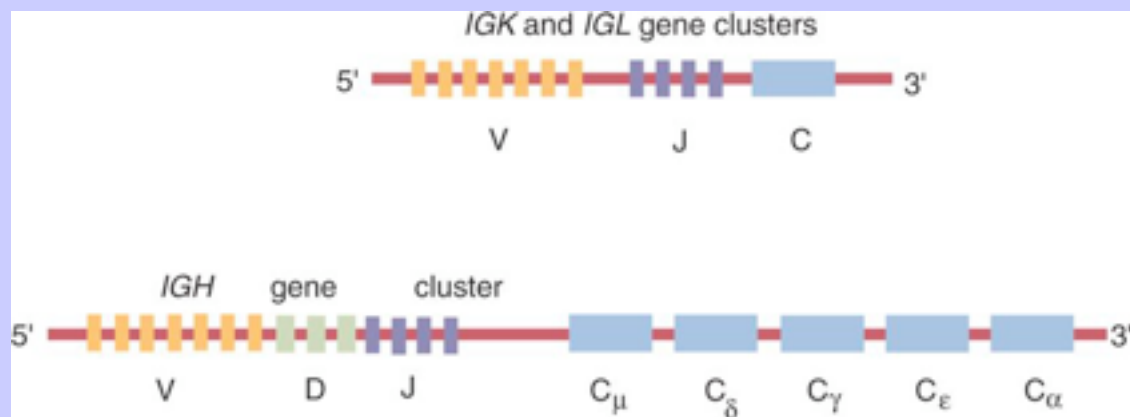
15.6 GENERATION OF JUNCTIONAL DIVERSITY

15.6.1 Gene Rearrangement

The most obvious way to generate V-region diversity is to randomly select one V gene from the available pool and join it to one randomly selected J gene—a process called recombination. Since many different V and J genes are available, the number of possible combinations can be very large. For example, if there are 100 V genes and 10 J genes, then $100 \times 10 = 1000$ different V regions can be constructed.

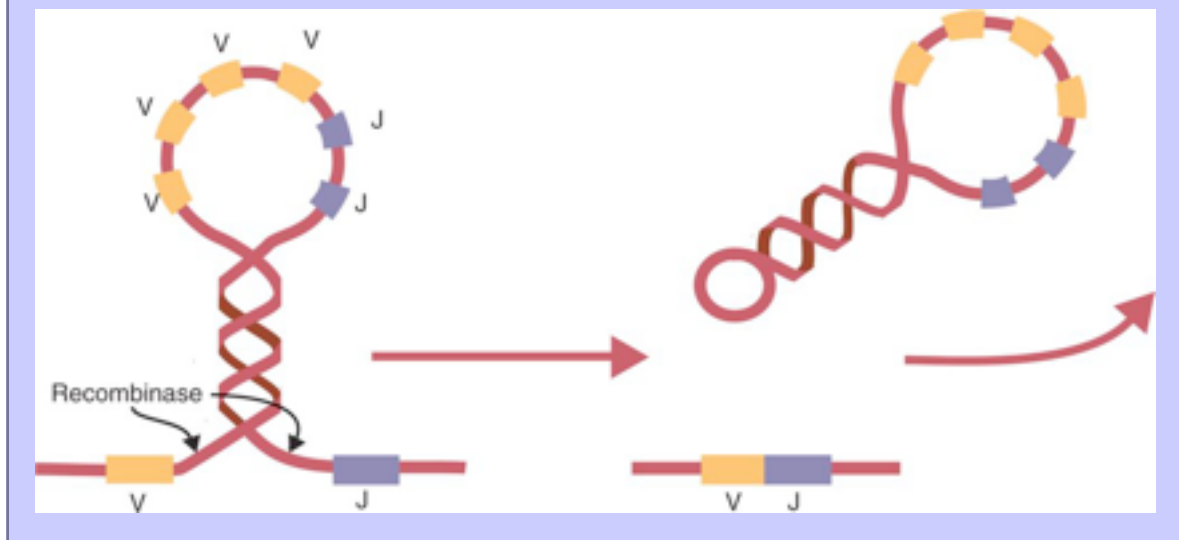
Light chain assembly requires the combination of one V, one J, and one C gene. During B cell development, the intervening genes are looped out, excised, and discarded. The V and J genes have sites at each end that guide the cutting enzymes ([Figure 15-4](#)). The

FIGURE 15-3 Genes coding for immunoglobulin light chains and heavy chains. Note that there are two distinct clusters of light chain genes, one coding for kappa chains and one coding for lambda chains. These are located on different chromosomes. The precise number of V, D, and J genes varies among species.



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FIGURE 15-4 One of the most important mechanisms for deleting unwanted genes is looping out. In this case, unwanted V genes form a loop that is then cut off and the cut ends joined together. As a result, the desired V gene is linked directly to a J gene.



looped-out genes are chopped off and the free ends of the DNA are rejoined so that the genes form a continuous sequence that leaves a V gene attached directly to a J gene. Two sets of enzymes are used in this process. Recombinases cut the DNA at two points, excising unwanted gene segments. Following this, DNA repair enzymes join the two free ends to form a continuous sequence. If any of these enzymes are defective, antibodies (and TCRs) cannot be made. In foals with severe combined immunodeficiency, for example, there is a defect in the DNA repair enzyme that joins the cut ends. As a result, these foals cannot make either TCRs or BCRs and so have no functional B cells or T cells (see [Chapter 34](#)).

Light chain gene recombination occurs in two stages. Randomly selected V and J genes are first joined to form a complete V-region gene. The joined V-J genes remain separated from the C gene until messenger RNA is generated. The complete V-J-C mRNA is then translated to form a light chain ([Figure 15-5](#)).

When a heavy chain V region is assembled, the situation is complicated by the presence of the D genes between the V and J genes. Thus construction of this V region requires the splicing together of *IGHV*, *IGHD*, and *IGHJ* genes ([Figure 15-6](#)). This use of three randomly selected genes enormously increases the amount of variability. For example, if a pool of 100 V, 10 J, and 10 D genes are recombined, then $100 \times 10 \times 10 = 10,000$ different V regions can be constructed. The recombination of these genes also occurs in a specific order. Thus *IGHD* is first joined to *IGHJ* and *IGHV* is added last. After transcription, any remaining unwanted segments are deleted and the complete V-D-J-C mRNA is translated to form a heavy chain.

Although the random selection of genes from two or three different pools generates a large number of different combinations, not all of these combinations will produce usable antibodies. Some combinations may result in a nucleotide sequence that cannot be translated into protein. These are called nonproductive rearrangements. For example, nucleotides are read as triplets called codons, each of which codes for a specific amino acid. If the codons are to be read correctly, the sequence must be in the correct reading frame. If bases are inserted or deleted so that the codon reading frame is changed, the resulting gene may code for a totally different amino acid

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sequence. If this frameshift results in inappropriate splicing, translation is prematurely terminated. It is probable that nonproductive rearrangements are produced two out of three times during B cell development. When this happens, the B cell has several additional opportunities to produce a functional antibody. For example, immature B cells initially use one of the *IGK* genes (Figure 15-7). If this fails to produce a functional light chain, they switch to the other *IGK* allele for a second attempt. If this does not work, the B cell will use one of the *IGL* alleles, and if this fails, the second *IGL* allele represents the last resort. If all these efforts fail to produce a functional light chain, the B cell cannot make a functional immunoglobulin. It will die without participating in an immune response.

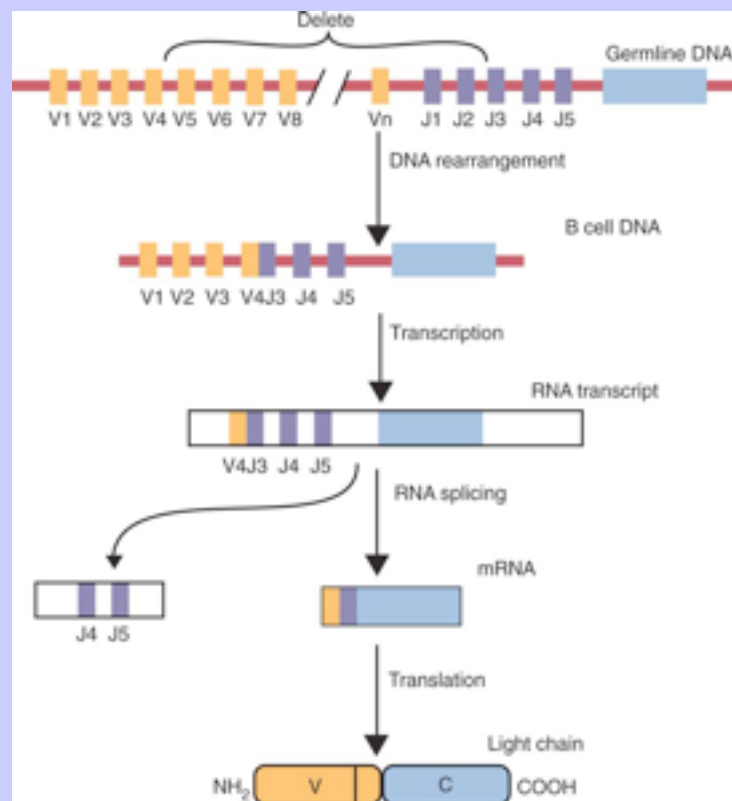
The sequence of events described above has been worked out in mice and humans and may not apply to domestic mammals. One obvious difference lies in the use of κ and λ light chains. In mice, rabbits, pigs, and humans, κ chains are preferentially used (95% in mice, 90% in rabbits, 60% in pigs, 60% in humans). In the other domestic species, λ light chains predominate (98% in ruminants, 60% to 90% in horses). The reasons for these differences are unknown.

It should also be pointed out that immunoglobulin gene rearrangement is not entirely random. For

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FIGURE 15-5 Construction of an immunoglobulin light chain. V and J genes are first joined. The VJ and C genes remain separated until RNA splicing occurs. DNA rearrangement occurs during early B cell development so that each individual B cell is committed to making a single form of light chain for its antigen receptor.



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example, in rabbits, mice, and humans the most 3' *IGHV* genes tend to be used most often. This preferential use of certain genes results from a combination of factors including the recombination signal sequences, the accessibility of the genes to the recombinase enzyme, sequences at the splicing sites, and cellular selection by antigen.

15.6.2 Base Deletion

Although random recombination of genes generates much V-region diversity, additional mechanisms can increase this diversity still further. For example, endonucleases remove bases from the cut ends of the genes. As a result, the precise nucleotide at which V and J genes join can vary, leading to changes in the base sequence at the splice site. This results in variations in the amino acid sequence in the V region.

15.6.3 Base Insertion

In immunoglobulin heavy chain gene processing, additional bases may be inserted at the V-D and D-J splice sites. Some of these nucleotides (N nucleotides) are added randomly by an enzyme called terminal deoxynucleotidyltransferase (TdT). Consequently, between 1 and 10 N-nucleotides may be inserted between V and D and between D and J.

15.6.4 Receptor Editing

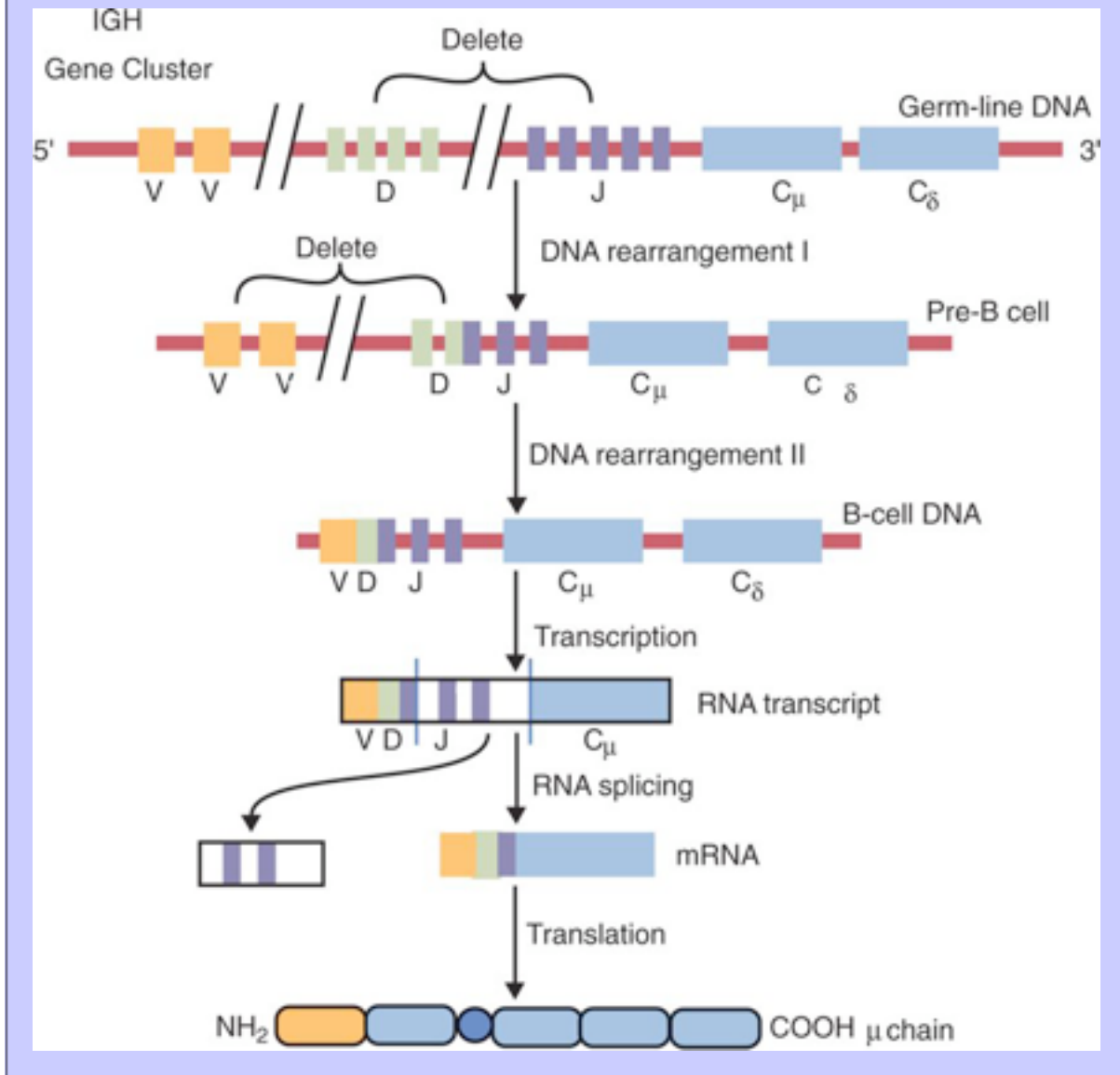
Although each new B cell expresses a distinct antigen receptor, developing B cells can continue to rearrange their V, D, and J genes even after exposure to antigens. Thus a B cell expressing a specific κ chain may restart V-gene rearrangement by switching to the other *IGKV* genes or even switching to either of the *IGLV* genes. It can continue to rearrange upstream, nonrearranged V genes or downstream nonrearranged J genes. This receptor editing, which occurs within germinal centers, may be a method of eliminating receptors that bind to self-antigens (see [Chapter 31](#)).

15.7 SOMATIC MUTATION

Although gene recombination can generate many diverse antigen receptors in immature B cells, antibodies produced early in an immune response may

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FIGURE 15-6 Production of a complete immunoglobulin heavy chain gene. Two DNA rearrangement events are required to link V, D, and J genes together.



bind antigens relatively weakly. In addition, gene recombination cannot account for all the variability that occurs in immunoglobulin V regions. For example, there are three CDRs within a V region ([Figure 15-8](#)). One of these, CDR3, is found around position 96 and clearly results from gene recombination. However, CDR1 and CDR2 are located far from V-J or V-D-J splice sites. Other mechanisms of generating antibody variability must exist ([Box 15-1](#)). In fact, gene recombination is only the first step in generating antibody diversity. It is followed by mechanisms that can generate antibodies that bind very strongly and specifically to an antigen. Thus the antibody response must be “fine tuned,” and this is driven by somatic mutation.

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Following initial exposure to an antigen, B cells form germinal centers. The B cells proliferate in the dark zones of germinal centers and then undergo antigen-driven selection in the light zones. If antibodies are isolated at intervals after immunization and their V region sequences studied, progressive changes are seen as the immune response progresses. These changes result from mutations within the recombined *IGHV* genes.

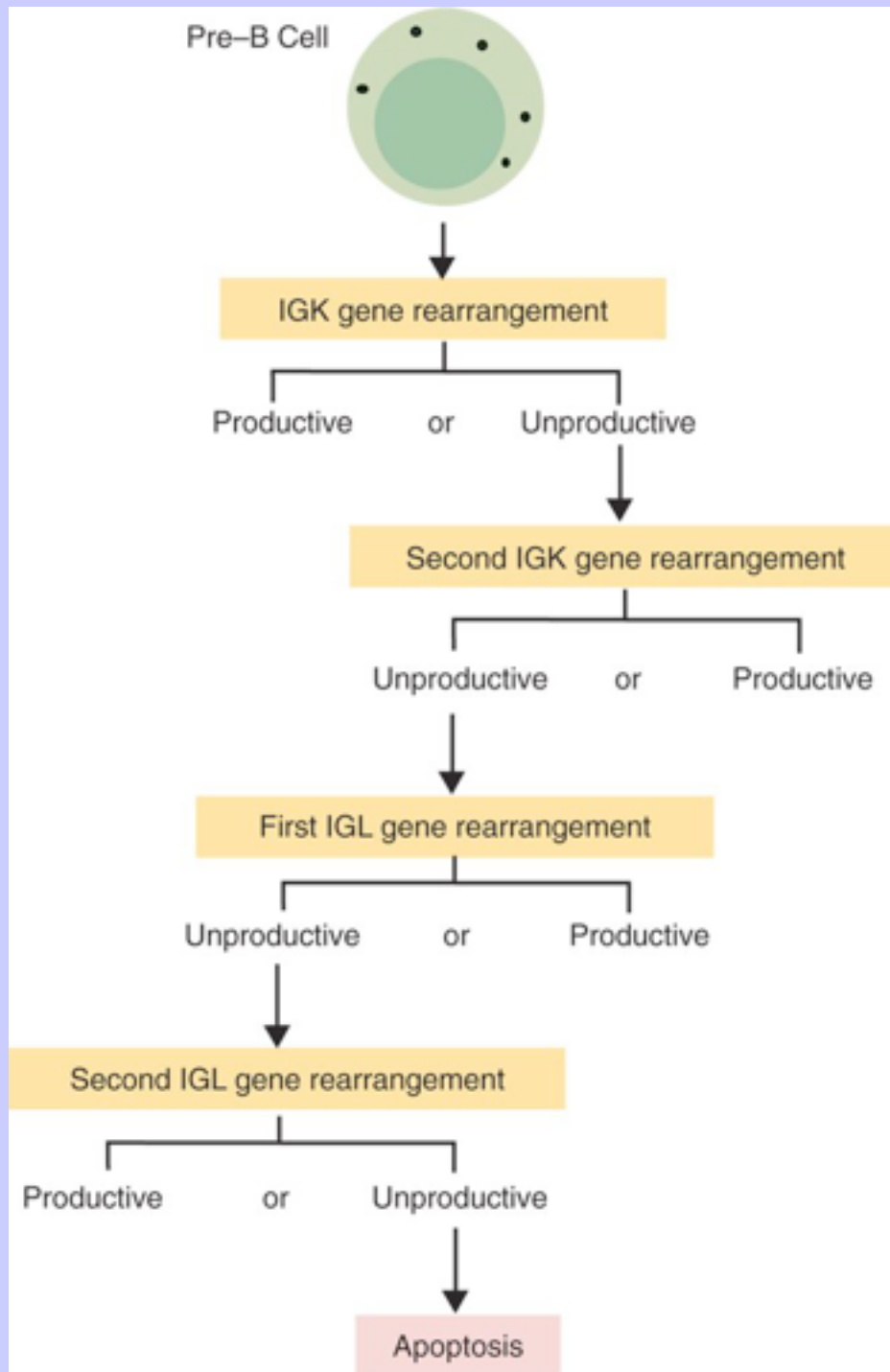
Random mutations in immunoglobulin V regions are triggered by antigen cross-linking of two BCRs, by the binding of CD40 to CD154, and by the binding of CD80 to CD28. These signals activate a B cell enzyme called cytidine deaminase. This enzyme deaminates the cytidines in V region DNA and so converts them to uracils. These uracils are recognized as errors (after all, uracil is not normally found in DNA), and their presence therefore triggers repair processes. One of these repair processes employs a DNA polymerase that mistakenly replaces the uracils with thymines during transcription. Other mechanisms delete the uracils and leave a gap that is repaired by DNA polymerases using randomly selected nucleotides. Thus the gaps are “patched” by short nucleotide sequences. As a result of this “repair,” V-region gene sequences change as the B cells respond to antigens. On average, one amino acid changes each time a B cell divides.

The degree to which a B cell is stimulated is directly related to the strength (affinity) with which a receptor binds an antigen. Any B cells whose newly modified BCRs cannot bind the antigen will no longer be stimulated and so will die. In contrast, those B cells whose receptors can bind the antigen with a higher affinity will be preferentially stimulated. They will survive and proliferate ([Figure 15-9](#)). The better the fit, the greater will be their stimulus to divide. Thus while responding to an antigen, successive cycles of mutation and selection lead progressively to the generation of populations of B cells producing very-high-affinity antibodies.

Somatic mutation does not begin until after B cells have switched from making IgM to making either IgG or IgA. This suggests that the mutation mechanism

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FIGURE 15-7 The B cell has four attempts to make a functional immunoglobulin. If it fails in all four attempts, the cell undergoes apoptosis.



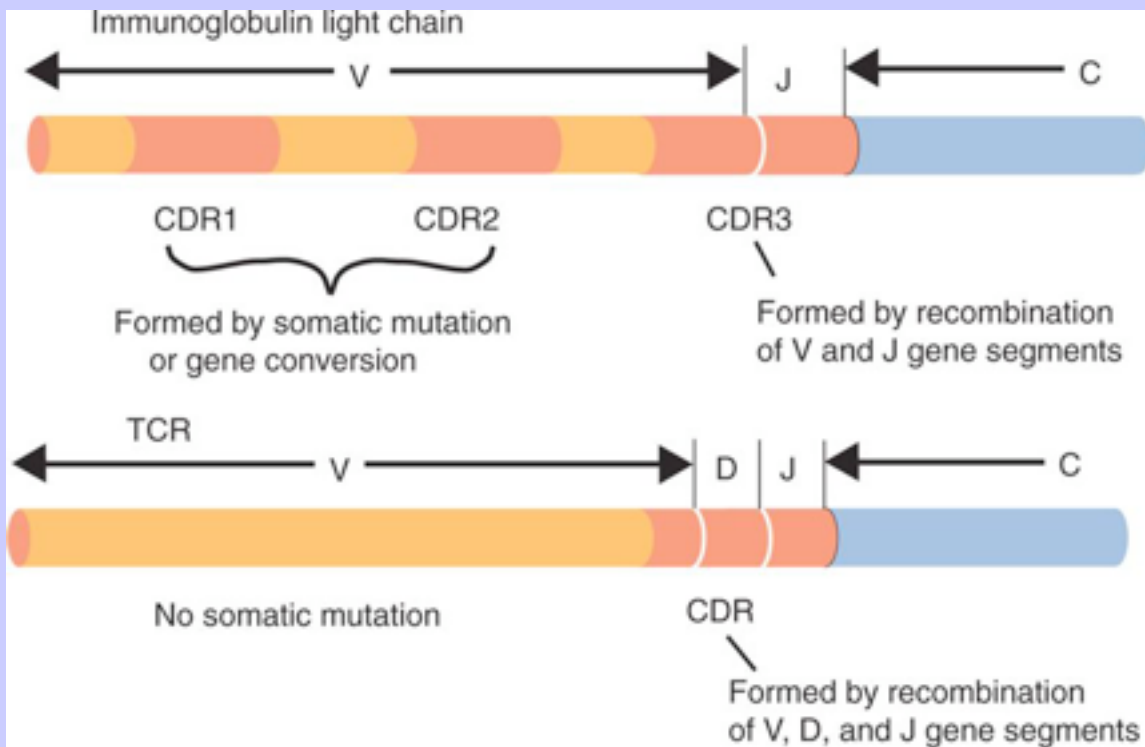
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is not activated until after a responding B cell had become committed to utilizing a specific heavy chain V gene. This explains why the affinity of IgM antibodies does not increase during an immune response whereas the affinity of IgG antibodies does.

15.8 GENE CONVERSION

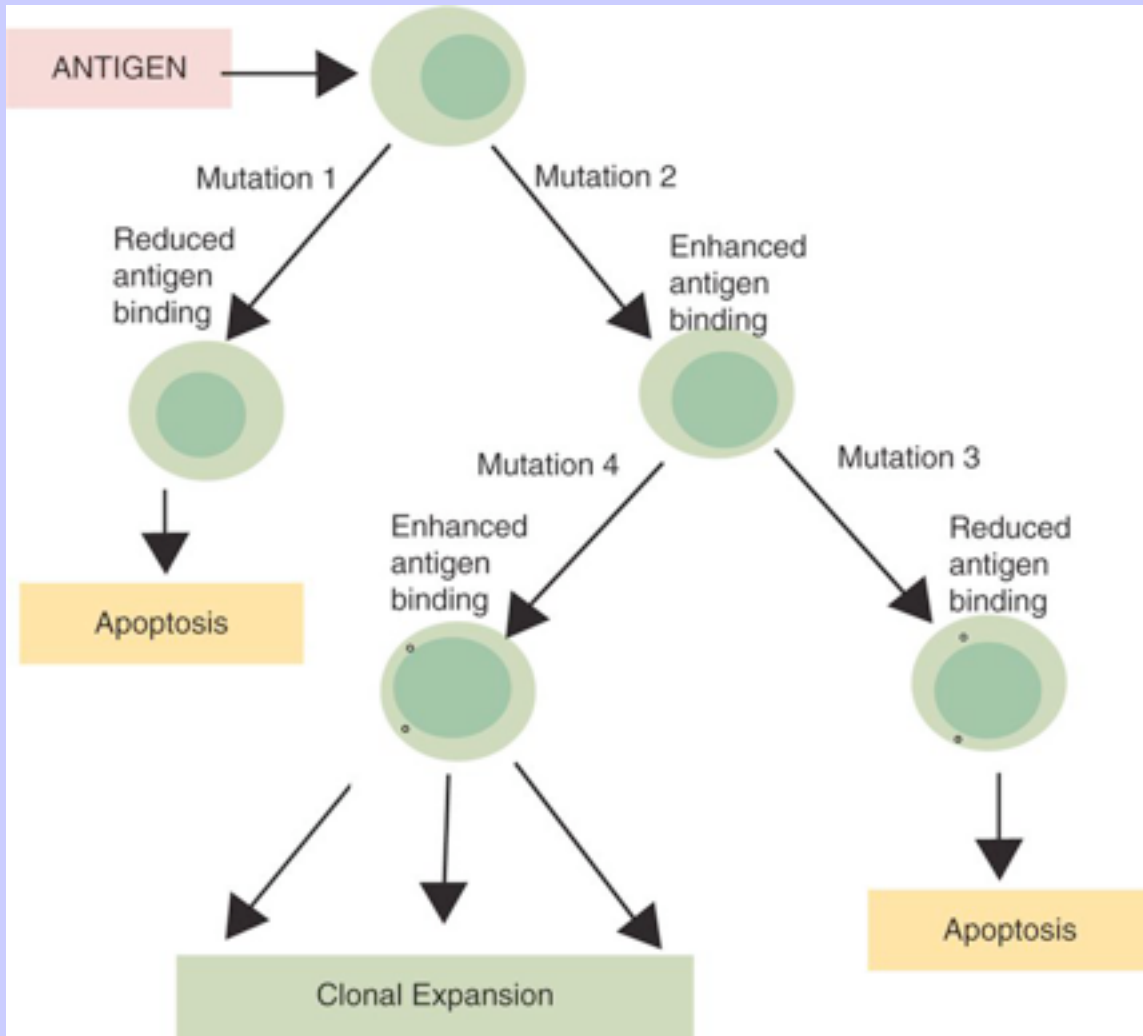
In mammals other than the human and mouse, there may not be a lot of V gene diversity. As a result, gene recombination cannot explain immunoglobulin diversity. In these species, V-region diversity is generated by a process called gene conversion ([Figure 15-10](#)). Species that employ gene conversion must have available either multiple V genes or pseudogenes. (Pseudogenes are segments of DNA that are defective and so cannot be transcribed.) During gene conversion, B cell cytidine deaminase inserts a uracil that is then removed, leaving a gap in the nearest V gene. The gap is then filled by randomly selected short segments

FIGURE 15-8 The major difference between the variable regions of the T cell receptor (*TCR*) and immunoglobulins is in the formation of complementarity-determining regions (*CDR*). Immunoglobulins have three CDRs. CDR1 and CDR2 are generated by somatic mutation. CDR2 is generated by gene conversion. This option is not available to the TCR, where somatic mutation is stringently avoided.



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FIGURE 15-9 Selection of somatic mutants. Spontaneous mutation during the expansion of a B cell clone results in the development of cells with B cell receptors that differ in their ability to bind antigen. Cells that bind antigen strongly will be more intensely stimulated than cells that bind it weakly.



obtained from one or more of the upstream V-region genes or pseudogenes. The “repaired” V gene will therefore have a different sequence than its precursor. Some of these gene conversion events produce an “inactive” V gene that cannot make a functional V region. In these cases, affected B cells are eliminated. B cells with active V genes, on the other hand, bind antigen, divide, and differentiate.

15.8.1 Box 15-1 Methods of Generating Antibody Diversity

VJ and VDJ gene recombination

Base deletion

Base insertion

Somatic mutation

Combinatorial association

Gene conversion

Receptor editing

15.8.2 Receptor Assembly

When B cell antigen receptors are generated, the assembly process occurs in a consistent order. The first chain to be assembled splices V, D, and J genes together. This chain is capable of generating much more junctional and combinatorial diversity than the other and so contributes the major portion of receptor diversity. Examples include the heavy chain in B cells and the β and δ chains of the TCR. Initially, therefore, precursor B cells express only an immunoglobulin heavy chain. This heavy chain is linked to signal transduction molecules and a surrogate partner chain is provided so the pre-B cell can respond in a limited way to antigens. As a result a small clone of B cells expressing only an IgH chain is formed. Likewise pre-T cells can form an initial receptor consisting of only β or δ chains together with a surrogate partner chain. Signaling through this prereceptor triggers limited proliferation.

This initial phase is followed by assembly of a partner chain. In B cells this is the light chain; in T cells this is the α or γ chain. The partner chain uses only a single splice site between V and J and so contributes much less diversity to the antigen receptor. Once assembled, a complete BCR or TCR is formed.

Once a complete first chain is formed using VD and J genes, signaling mechanisms tend to prevent further recombination and rearrangement of its genes and so do not permit assembly of the second allele. The presence of the first chain also influences the generation of diversity in the partner chain. As a result, diversity generation in the second chain appears to “fine-tune” the antigen-binding abilities of the first chain.

15.8.3 Potential Immunoglobulin Diversity

The processes of gene rearrangement described above can generate enormous V-region diversity and antigenic specificity in several ways. First, as occurs in the case of the human *IGKV* family, only one of a possible 80 V genes is selected for transcription, as is only one of the five *IGKJ* genes. This random joining of segments will

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provide significant variability since there are 400 (80 × 5) possible *IGKV-IGKJ* combinations. The use of a third randomly selected gene segment will increase sequence diversity still further. Thus with 300 *IGHV*, 5 *IGHD*, and 2 *IGHJ* segments employed, as many as 3000 (300 × 5 × 2) different heavy chain V regions can be generated in humans. Since both heavy and light chains are used to form the antigen-binding site, the total number of possible combinations in humans is 1.2 million (400 × 3000). In addition, the presence of two splice sites multiplies the potential for diversity generated as a result of base deletion and insertion. However, as pointed out above, many of the gene combinations so formed may be of little functional use.

Taking all possible mechanisms into account, the number of different antigen-binding sites and hence binding specificities generated is about 1.8×10^{16} without accounting for somatic mutation. (This figure may be compared with the estimated 1×10^7 different antigens that the immune system can recognize.)

15.9 SPECIES DIFFERENCES

Mechanisms of antigen receptor diversity generation fall into two distinct patterns depending on species. Some mammals rely on gene recombination followed by somatic mutation. In these species, immunoglobulin diversity is continuously generated from B cell precursors throughout an animal's life. Other species, in contrast, use gene conversion for a short period early in life. After initial B cell diversity is generated, this pool of B cells expands by a self-renewing mechanism with little somatic mutation.

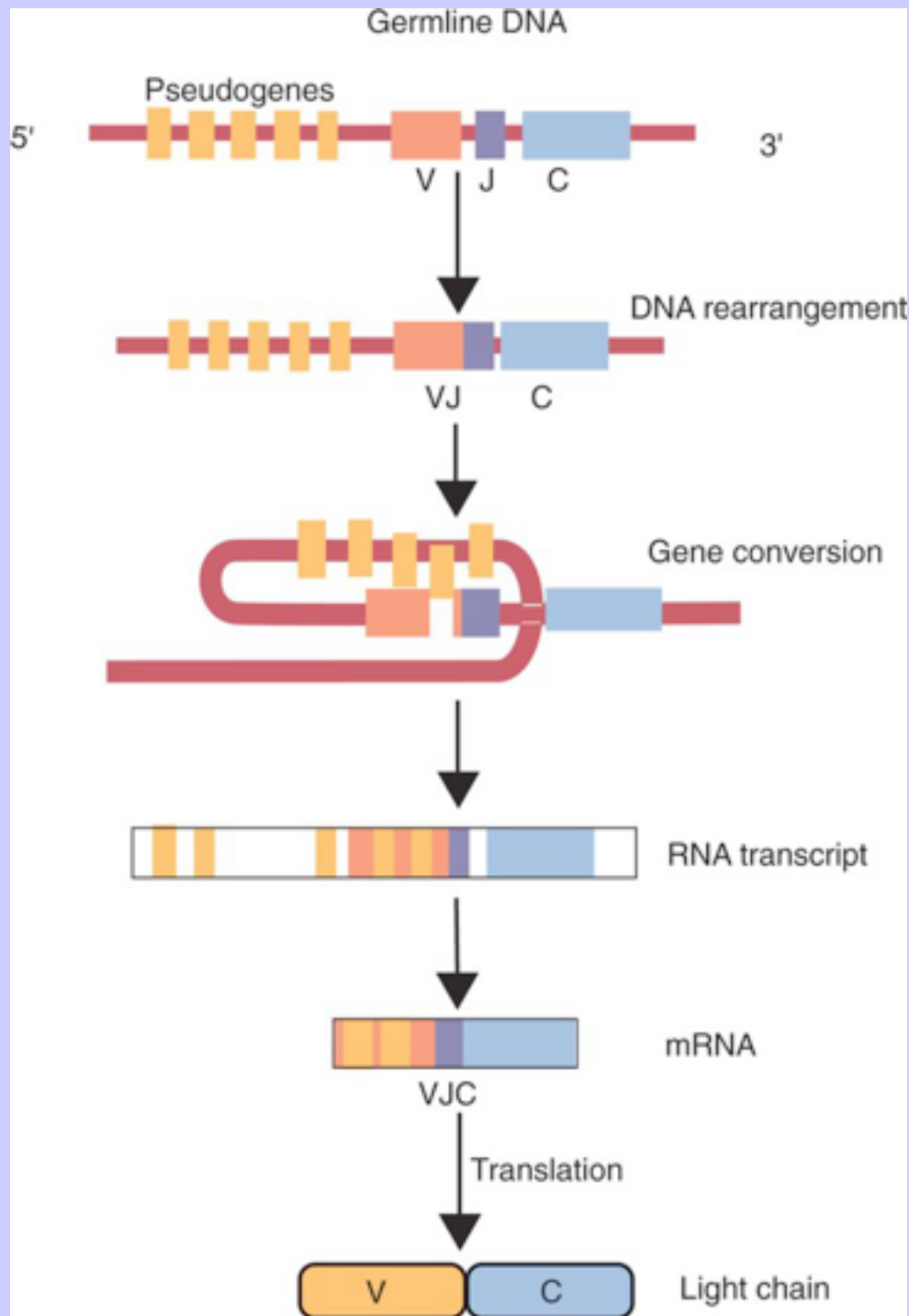
15.9.1 Cattle

Cattle likely use both processes to generate diversity. Thus they employ gene recombination for their light chains and gene conversion for their heavy chains. Initial diversification occurs in lymphoid organs followed by somatic mutation in ileal Peyer's patches. Cattle may have 15 closely related *IGHV* genes, all of which belong to a single family (Table 15-1). Cattle also have many V pseudogenes and both long and short *IGHD* genes. As a result, their heavy chain CDR3 regions are variable in size and in extreme cases may contain as many as 61 amino acids.

15.9.2 Sheep

Sheep also use both processes. Immature B cells first diversify their V (D) and J genes in lymphoid tissues such as the spleen or bone marrow. The immature cells then migrate to follicles in the ileal Peyer's patches, where additional diversification occurs as a result of somatic mutation (Figure 15-11). The initial diversification step is mediated by a mixture of mechanisms. Sheep light chain genes have more than 90 *IGLV* genes and a single *IGLJ* gene so that they are diversified through gene recombination. On the other hand,

FIGURE 15-10 Process of gene conversion. In this process, segments of upstream genes or pseudogenes are inserted into a single V region to generate sequence diversity.



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sheep have only a limited number of *IGHV* genes and therefore use gene conversion to diversify their heavy chains. They have six *IGHJ* genes, two of which are pseudogenes. One of the active genes, *IGHJ1*, is used in 90% of heavy chains, suggesting that gene recombination is minimal. More than 98% of all rearrangement events are in frame, and there are few N- or P-nucleotides. Unlike rabbit, human, or mouse, stimulation by intestinal commensal bacteria is not absolutely necessary for V-gene diversification in sheep.

Table 15-1 Examples of Different Gene Usage in Mammals

Species	IGKV	IGKJ	IGLV	IGLJ	IGHV	IGHJ	IGHD
Horse	20	5	25	4	>7	5	10
Bovine			20	4	15	2	3
Sheep	10	3	>100	1	7	2	>1
Pig	250	>5	100	3	20	1	2
Mouse	111	9	14	3	200	7	18
Human	48	9	69	8	215	27	30

15.9.3 Pigs

Pigs have about 20 *IGHV* genes, two *IGHD* genes, and a single germ line *IGHJ* gene. Early in fetal life the pig uses only 4 or 5 *IGHV* genes and their early repertoire consists of only 8 to 10 combinations. Later in fetal life this restricted repertoire is compensated for by early TdT activity and extensive, in frame, N-region addition leading to significant junctional diversity. Pig B cells do not undergo receptor editing because they have only one *IGHJ* gene.

The presence of commensal bacteria within the intestine significantly assists the development of pig B cells, whose numbers increase greatly during the first 2 weeks of age although receptor diversity may not increase significantly until 4 to 6 weeks of age. Germ-free pigs have serum immunoglobulin levels 20-fold to 100-fold less than conventional pigs. Conventional pigs exhibit much greater diversity in their mucosal IgM and IgA V genes (but not splenic IgG V genes) than germ-free pigs.

15.9.4 Rabbits

Rabbits primarily generate antibody diversity by gene conversion. Thus immature rabbit B cells first join a small number of V (D) and J genes and then migrate to the appendix. These V genes are subsequently diversified by gene conversion and somatic mutation within the appendix germinal centers. The presence of commensal bacteria is necessary for this diversification to occur. Glycans and other pathogen-associated molecular patterns produced by these bacteria are required to provoke this antibody diversification and B cell growth. Rabbits actually have more than 200 *IGHV* genes, but almost 90% of V-region rearrangements employ the V gene closest to the D segment. The other V genes presumably serve as donors for gene conversion.

15.9.5 Humans and Mice

Humans and mice with many *IGHV* genes use multiple gene segment rearrangements to generate much of their antibody diversity ([Table 15-2](#)). Additional diversity is generated at junctions by base deletion and insertion. The

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final layer of diversity is generated in these species by somatic mutation. In these species, B cells with diverse antigen receptors are produced throughout an animal's life.

FIGURE 15-11 Lymphoid organs where gene recombination, gene conversion, and somatic mutation occur.

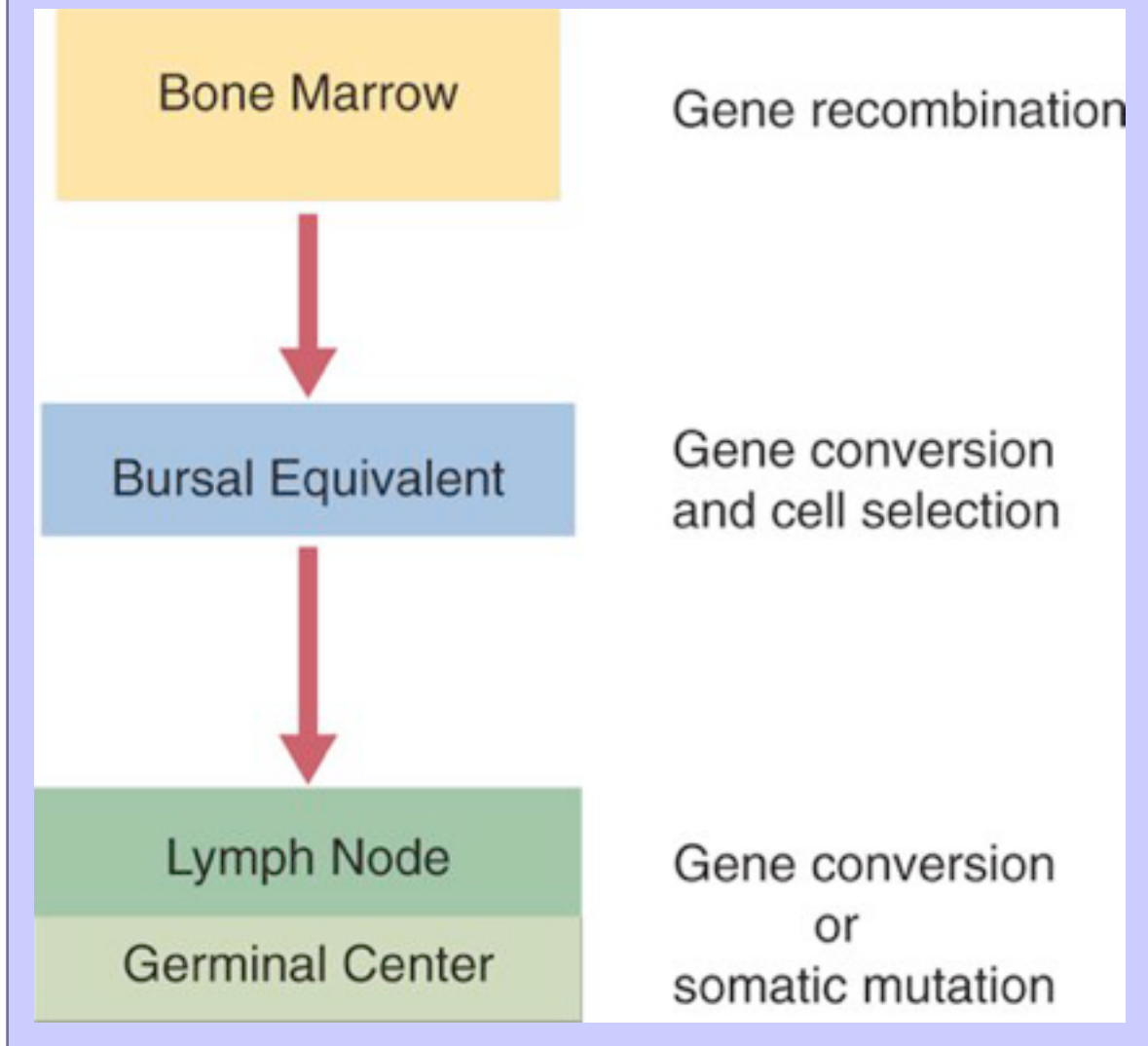


Table 15-2 Immunoglobulin Diversity among Mammals

Species	C _H Genes					C _L Genes		V _H and V _L Families		
	IgM	IgD	IgG	IgE	IgA	λ	κ	H	λ	κ
Horse	1	1	7	1	1	4	1	7	1	?
Bovine	2	1	3	1	1	4	1	1	2	?
Sheep	1	1	3	1	2	>1	1	1	6	3
Pig	1	1	8-12	1	1	1?	1	1	?	?
Dog	1	1	4	2?	1					
Rabbit	1	0	1	1	13	8	2	1	?	?
Mouse	1	1	4	1	1	3	1	14	3	4
Human	1	1	4	1	2	7	1	7	7	7

From Butler JE: *Scand J Immunol* 45:455-462, 1997 and other sources.

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15.9.6

Intestinal Bacteria and Expansion of the B Cell Repertoire

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As pointed out previously, some mammals, including the large domestic herbivores, develop their B cell antibody repertoire in two stages. The first stage involves diversification requiring rearrangements of a small number of V, D, and J gene segments. These B cells then migrate to the gut-associated lymphoid tissue (GALT), where they greatly increase the number of their B cells as well as the diversification of their B cell repertoire. Thus this second phase of B cell diversification takes place in intestinal lymphoid organs that are in direct contact with intestinal contents, and most especially the intestinal microflora. The importance of the microflora is supported by the failure of gnotobiotic (germ free) pigs to develop significant B cell diversity. Some of the intestinal microflora play an especially critical role in this process. For example, in rabbits, normal GALT development can take place in the presence of both *Bacteroides fragilis* and *Bacillus subtilis* but not by either alone. Other bacterial combinations are also effective, suggesting that some form of bacterial interaction is needed for optimal effect.

It has been demonstrated that a glycan from *B. fragilis* is processed by antigen-presenting cells and stimulates the growth, maturation, and cytokine production of CD4⁺ T cells. These T cells in turn stimulate the complete development and maturation of B cells.

Analysis of the expansion of intestinal B cells by commensal bacteria shows that it tends to affect B cells with certain V_H domains. Thus the expansion is not simply a specific response to microbial antigens but a polyclonal, non-antigen specific response. It may be directed through pattern recognition receptors such as the TLRs or as a result of superantigens binding to the BCR, or some combination thereof. The superantigen could be a specific microbial component, or it could be produced in host tissues under the influence of the bacteria. The preferential stimulation of B cells with specific V_H genes points to a bacterial super-antigen as the cause.

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15.10 T CELL RECEPTOR DIVERSITY

TCR and immunoglobulin gene rearrangements are specific in that immunoglobulin genes are not rearranged in T cells and TCR genes are not rearranged in B cells. Like immunoglobulins, the four peptide chains, α , β , γ , and δ , that make up the two types of TCR can bind many different specific antigens. They are able to do this because they each consist of a variable region attached to a constant region. The diversity of the TCR V region is generated only by gene recombination and combinatorial association in all species examined to date, unlike the diverse mechanisms employed by B cells.

15.10.1 T Cell Receptor Gene Structure

The four T cell receptor peptide chains are coded for by three gene clusters. The *TRA/D* cluster codes for both α and δ chains, the *TRB* cluster codes for β chains, and the *TRG* cluster codes for γ chains. All three clusters contain V, J, and C genes, and the *TRB* and *TRD* clusters also contain D genes ([Figure 15-12](#)). Each TCR cluster contains two or more C genes. In the *TRA/D* cluster the two C genes are functionally and structurally different, so that one codes for *TRAC* and the other for *TRDC*. The number of *TRGC* genes varies among species. Thus there are two different *TRGC* genes in humans, three in mice, four in pigs, and five in cattle and sheep. There are two identical *TRBC* genes in humans and mice. Some helper and cytotoxic T cells rearrange and express *TRA* and *TRB* genes (α/β T cells), whereas others use *TRG* and *TRD* genes (γ/δ T cells). As pointed out in earlier chapters, the functions of these two T cell populations vary among species.

15.10.1.1 α and δ Chains

The *TRA/D* cluster is unusual in that the *TRD* genes are embedded within the *TRA* cluster. When T cells are immature, they use δ chains for their antigen receptors. As the T cells mature, they delete the *TRD* genes and begin to use the α chain. Some V genes in the α/β cluster can participate in the assembly of both α and δ TCR chains.

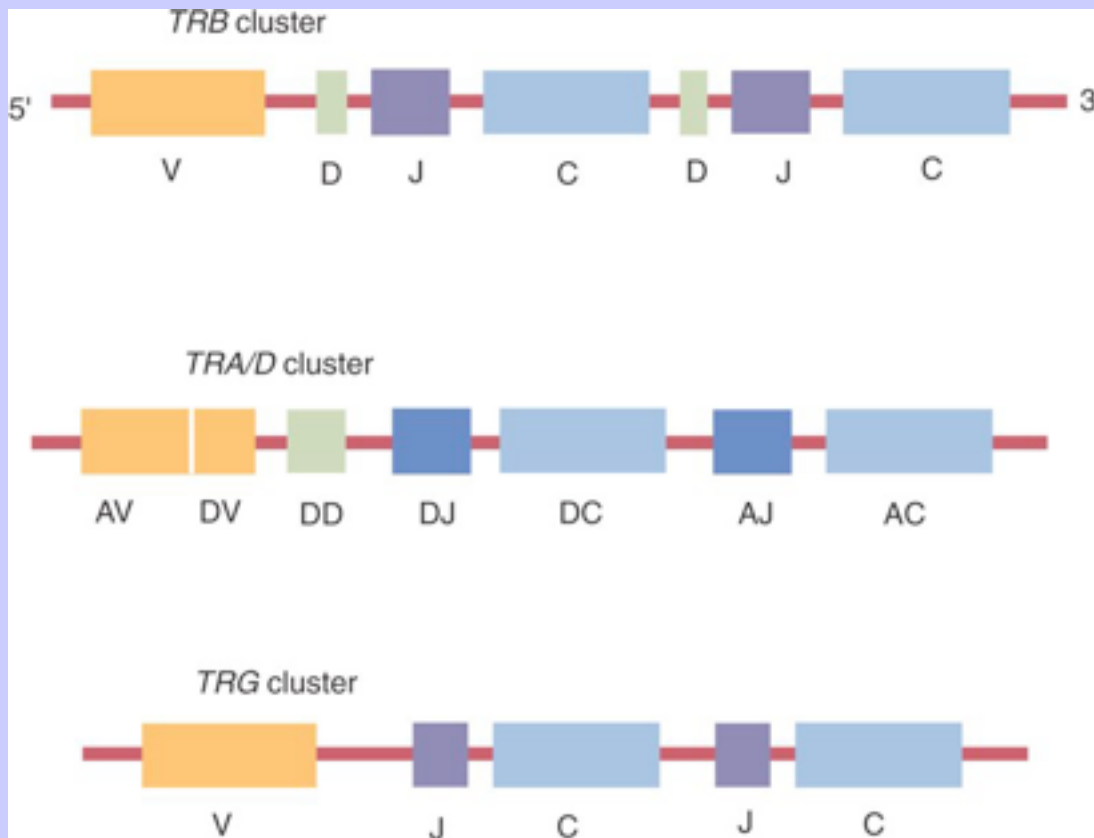
The *TRA* cluster contains V, J, and C genes divided into two regions by the embedded *TRD* genes. There are about 100 *TRAV* genes, about 75 *TRAJ* genes, and a single *TRAC* gene in humans. Thus there are many more J genes here than are found in the immunoglobulin gene clusters. The *TRD* cluster contains V, D, J, and C genes. In mice there are about 10 *TRDV* genes, 2 *TRDD* genes, 2 *TRDJ* genes, and 1 *TRDC* gene. Both *TRDD* genes may contribute to the final product so that a complete Vd sequence may be coded for by *TRDV*, *TRDD1*, *TRDD2*, and *TRDJ* genes. This arrangement can generate much greater diversity than the other receptor chains. In pigs there are 61 *TRAJ* genes, 31 *TRDV* genes, 3 *TRDD* genes, and 4 *TRDJ* genes. There are 24 *TRDV* genes in sheep and 12 in cattle. Both have 3 *TRDJ* genes, but *TRDD* genes have not been described in these species.

15.10.1.2 β Chain

The *TRB* cluster contains a large number of V genes located upstream of two nearly identical D-J-C clusters,

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FIGURE 15-12 Basic structure of the three gene clusters that code for the four different T cell antigen receptor chains. The genes for δ chains are embedded within the α -chain genes to form a single cluster.



each containing about six functional J genes. The D genes are all similar in sequence and length, and their use is optional. Any of the *TRBV* genes may be joined to either of the two D-J-C genes, and the V gene may join to either a D or a J gene. Dogs have about 20 *TRBV* genes, but about one third of these make up 90% of the T cell repertoire. TCR V-gene usage may in fact be restricted to a single V-gene family in the dog.

15.10.1.2.1 Box 15-2 Methods of Generating TCR Diversity

VJ, VDJ, and VDDJ recombination

Base deletion

Base insertion

Combinatorial association

15.10.1.3 γ Chain

The *TRG* cluster in humans contains about 50 V genes, 5 J genes, and 2 C genes. Horses also possess 2; mice, pigs, and cattle, 6; and sheep, 5 *TRGC* genes. There is no *TRGD* gene, so that *TRGV* genes combine directly with *TRGJ* genes. Cattle possess 11 *TRGV* genes.

15.10.2 Generation of T Cell Receptor V-Region Diversity

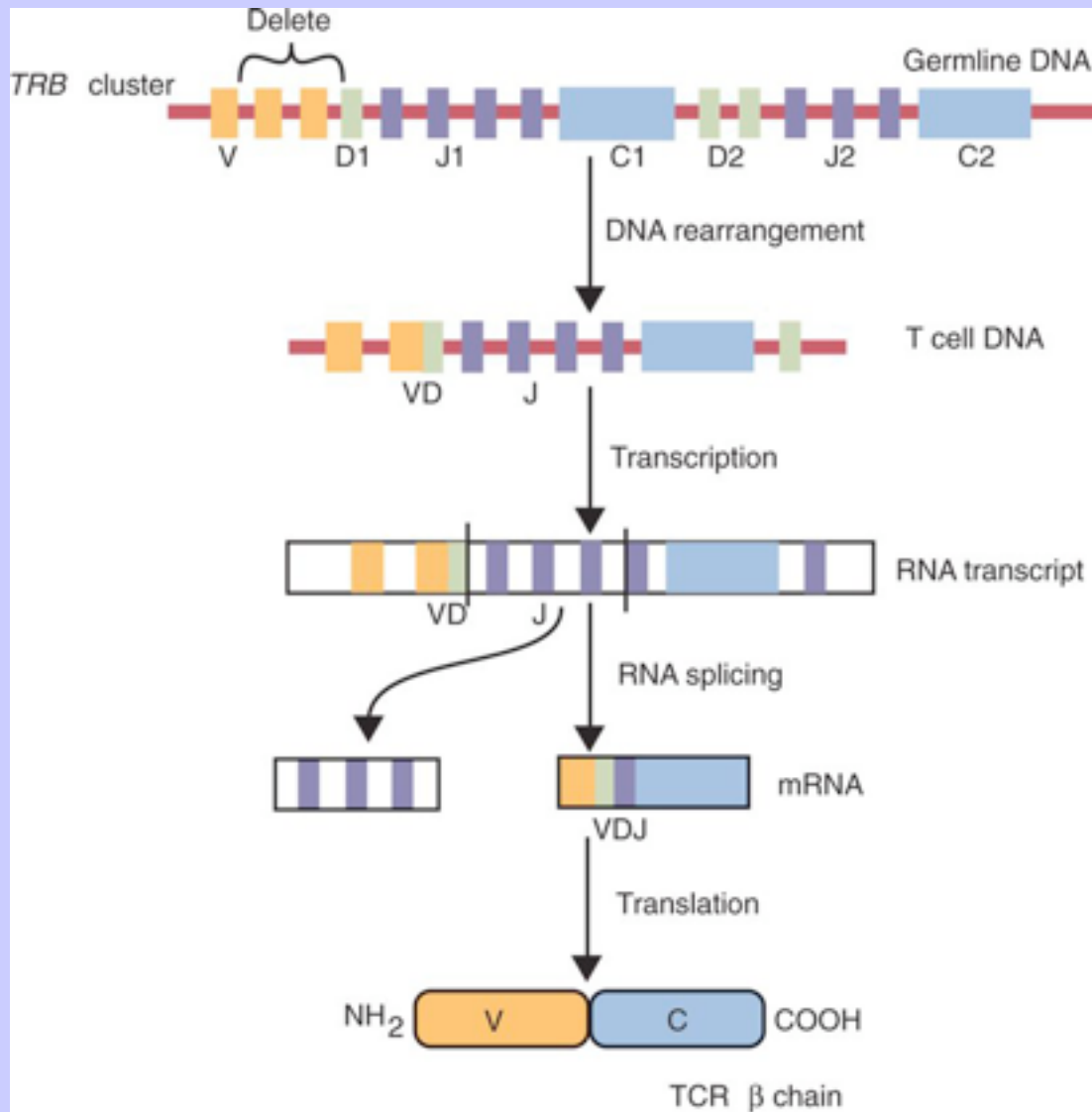
There are three hypervariable regions (CDRs) in each TCR V region. The first two, located within the V genes, have probably arisen through selection of V genes. The third is encoded in the region where V, D, and J genes recombine. The greatest diversity in the TCR chains is generated by the recombination of multiple V, J, and D genes ([Box 15-2](#)). Neither somatic mutation nor gene conversion occurs in TCR genes. Thus the various genes that are separate in the germ line are brought together by DNA rearrangement, combinatorial association, and base insertion or deletion as T cells differentiate ([Figure 15-13](#)).

15.10.2.1 Gene Rearrangement

TCR α and γ chains use only V and J genes to form their V regions. TCR β and δ chains use V, D, and J genes to form their V regions. Mouse δ chains can use both of their D genes and, as a result, V-D-D-J constructs can be formed. In β and δ chains the reading frame of the D genes is commonly changed and can yield productive rearrangements. This is a rare event in immunoglobulins. Looping out and deletion account for more than 75% of TCR rearrangements. The remainder of the rearrangements is due to either unequal sister chromatid exchange or inversion, i.e., moving an inverted segment of gene into a position beside a segment in the opposite orientation. Looping out and deletion of TCR genes are mediated by signals identical to those of immunoglobulins. Thus the same joining enzyme (a recombinase) probably acts on both immunoglobulin and TCR genes.

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FIGURE 15-13 The production of a complete T cell receptor peptide chain. Note the similarities between this and [Figure 15-6](#).



15.10.2.2 Base Insertion and Deletion

Although in general TCRs are constructed from fewer V-, D-, and J-region genes than immunoglobulins, their diversity is greater as a result of junctional diversity. Thus random N-nucleotides may be inserted at the V, D, and J junctions as a result of the action of TdT. As many as five nucleotides can be added between V and D and four between D and J genes. Likewise, random nucleotides may be removed by nucleases. This insertion of N-nucleotides and base deletion is much more extensive than that seen in immunoglobulin genes and is probably the most significant component of TCR junctional diversity.

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15.10.2.3 Somatic Mutation

Somatic mutation does not occur in TCR V genes. Although T cells must be able to recognize a foreign antigen in association with the presenting major histocompatibility complex (MHC) molecule, it is essential that they recognize self-MHC molecules while not responding to self-antigens. If random somatic mutation were to occur, it would carry the unacceptable risk of altering MHC restriction, thus rendering the foreign antigen unrecognizable. It might also lead to the production of TCRs able to bind self-antigens and thus trigger autoimmunity.

15.10.3 Where Does This Happen?

TCR genes are rearranged and expressed in the developing thymus. The developing T cell first attempts to rearrange the genes for γ and δ and, if this is not productive, proceeds to rearrange those for α and β chains. (Alternatively, it has been suggested that γ/δ and α/β belong to two different cell lines and express these receptors independently.) Rearrangement in the *TRD* cluster appears to be the earliest event in T cell development. Because of the geometry of the *TRA/D* cluster, joining of a *TRAV* gene to a *TRAJ* gene inevitably deletes the D genes on that allele. Thus α -chain rearrangements eliminate any possibility of d-chain expression.

15.10.4 T Cell Receptor Diversity

In the human *TRA* locus there are 75 *TRAJ* genes and 100 *TRAV* genes, giving $75 \times 100 = 75 \times 10^2$ possible combinations. There is also N-region addition and base deletion, resulting in great junctional diversity. After correction for codon redundancy and correct reading frame, the number of potentially different TCR α chains is about 10^6 . In the human *TRB* locus there are about 75 *TRBV* genes, 2 *TRBD* genes, and 12 *TRBJ* genes giving $75 \times 2 \times 12 = 1800$ possible combinations. In addition, there is junctional diversity and the use of perhaps 110 possible different *TRBD* combinations. After corrections there are about 5×10^9 possible VDJb sequences. Thus the number of possible different TCR α/β combinations is $5 \times 10^9 \times 10^6 = 5 \times 10^{15}$. (A somewhat similar figure can be arrived at for the mouse. However, a mouse has only 5×10^7 T cells, so this is much more potential diversity than a mouse would ever be able to use.)

In the human *TRD* cluster, a combination of V-region diversity, two *TRDD* genes, three sites where N addition and deletion can occur, and diversity in V-J joining position can generate about 10^{14} possible amino acid sequences, whereas in *TRGV* 7×10^6 different sequences are possible. There is no difficulty, therefore, in accounting for the enormous diversity seen in the TCRs.

15.11 γ/δ T CELL DIVERSITY

The function of γ/δ T cells differs among mammalian species. For example, in humans and mice there are few V genes in the *TRD* and *TRG* loci and the combinational repertoire is relatively small. In addition, the γ/δ TCR repertoire is severely restricted since the cells bearing these receptors use only a few V-gene combinations. As a result, 70% to 90% of human γ/δ T cells express the *TRGV9* and *TRDV2* gene products. In contrast, human α/β T cells show a wide range of binding specificities. Thus in humans and mice there is a marked difference between the size of the α/β and γ/δ TCR repertoires. The α/β T cells recognize and respond to a wide variety of processed

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antigens. In contrast, γ/δ T cells probably have a limited role in defense and recognize a limited number of antigens in humans and mice.

The situation in artiodactyls is very different. In these mammals, γ/δ T cells form a much larger proportion of total T cells. For example, in young lambs or calves, they account for up to 60% of T cells. In addition, ruminant γ/δ T cells show a considerably greater receptor diversity. Thus in the sheep, γ/δ V-region diversity results from the use of 24 *TRDV* genes and 15 to 20 *TRGV* genes that contain two distinct hypervariable segments similar to the CDRs seen in immunoglobulin V genes. In addition, the sheep γ/δ heterodimer occurs in at least five forms made by the association of one Cd chain with one of five Cg chains. All this suggests that the sheep γ/δ T cells may recognize a very wide variety of antigens.

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¹⁶ CHAPTER 16 T Cell Function and the Destruction of Cell-Associated Invaders

^{16.1} KEY POINTS

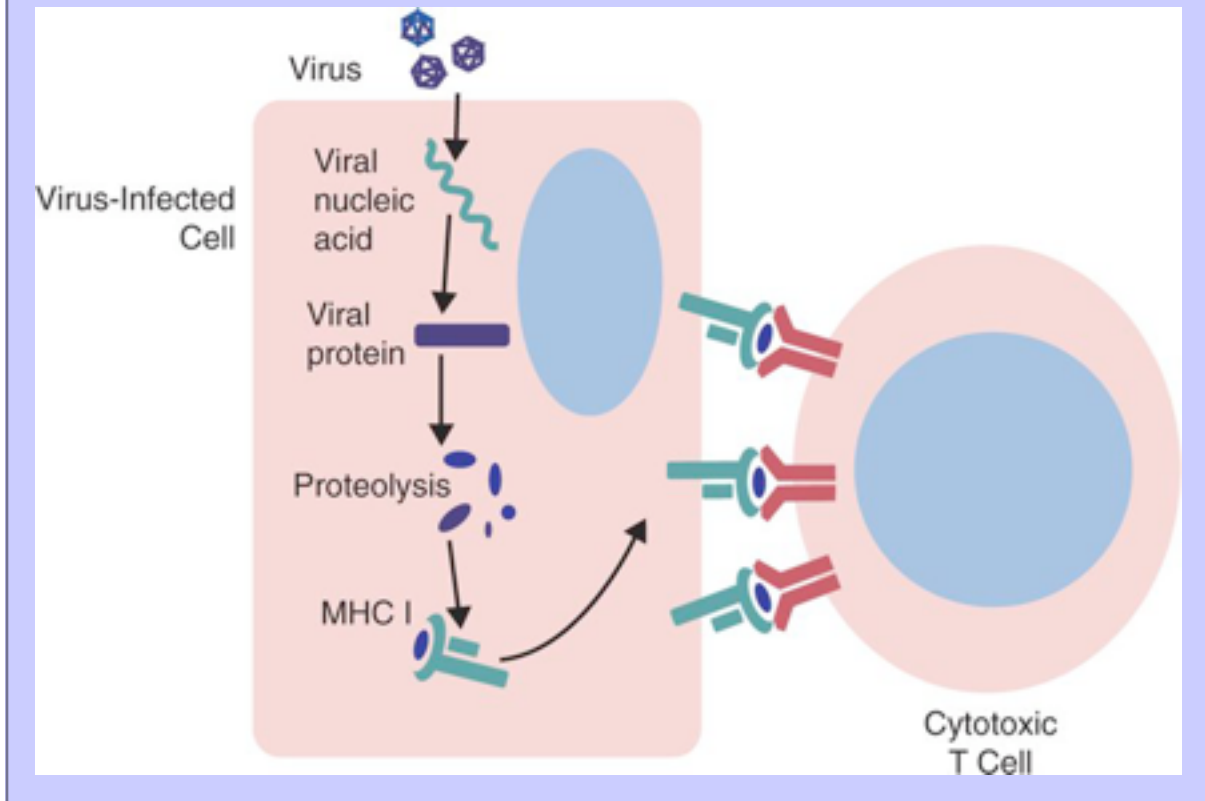
- Apoptosis is a mechanism whereby the body rids itself of unwanted cells. There are two major apoptotic pathways, intrinsic and extrinsic. Both result in the activation of an intracellular caspase cascade.
- Cell-mediated immunity eliminates abnormal cells and intracellular pathogens.
- One pathway of cell-mediated immunity involves the forced apoptosis of virus-infected target cells by cytotoxic T cells.
- The cytotoxic T cells use two mechanisms to kill targets. They may trigger the intrinsic pathway using perforins and granzymes. Alternatively, they may trigger the extrinsic pathway using the death receptor fas and its ligand.
- Intracellular organisms may also be killed by activation of M1 macrophages by interferon- γ produced by Th1 cells.

Antibodies bind to invading organisms in the circulation or tissue fluids, and so hasten their destruction. However, not all foreign organisms live outside cells. All viruses and some bacteria grow within cells in sites inaccessible to antibody. Antibodies are therefore of limited usefulness in defending the body against these invaders. Viruses and other intracellular organisms must be eliminated by other mechanisms. For this the body uses two different techniques. Either the infected cell is killed rapidly so that the invader has no time to grow or, alternatively, the infected cell develops the ability to destroy the intracellular organism. In general, organisms such as viruses that enter the cell cytoplasm or nucleus are killed by cytotoxic mechanisms whereas organisms such as bacteria or parasites that reside within cytoplasmic vacuoles are destroyed through cell activation. T cells mediate both processes. The antigens that trigger these responses arise from intracellular locations and are therefore called endogenous antigens.

^{16.2} ENDOGENOUS ANTIGENS

As described in [Chapter 9](#), every time a cell makes a protein, a sample is processed and small peptides are carried to the cell surface bound to major histocompatibility complex (MHC) class I molecules ([Figure 16-1](#)). If these peptides are not recognized by T cells, no response is triggered. If, however, the peptide-MHC complex binds to a T cell antigen receptor (TCR), that T cell is triggered to respond. Thus when a virus infects a cell, T cells may recognize many of the peptides derived from viral proteins. The T cells that respond to these endogenous antigens are CD8⁺. CD8 is the receptor T cells use to bind to MHC class I molecules.

FIGURE 16-1 Processing of endogenous antigen. Endogenous antigen is first broken down into small peptides and inserted into the antigen-binding groove of major histocompatibility complex (MHC) class I molecules. When presented on a cell surface, antigen bound to MHC class I molecules triggers a cytotoxic T cell response.



16.3 APOPTOSIS

Cells can kill themselves. Old, surplus, damaged, or abnormal cells that would otherwise interfere with normal tissue functions can be induced to die if and when necessary. This cell suicide is called apoptosis. Apoptosis is carefully regulated. The apoptotic machinery must only be activated when a cell must die. Structurally, apoptosis is characterized by membrane blebbing, nuclear fragmentation, and phagocytosis of the dying cell.

There are two major pathways of apoptosis. The “extrinsic” pathway is triggered by cytokines such as tumor necrosis factor- α (TNF- α) acting through specific “death” receptors such as CD95 (fas). In cells dying by this pathway, ligand binding to death receptors leads to the formation of a death-inducing signaling complex (DISC) containing caspase 8 and caspase 10. The “intrinsic” pathway is triggered by activation of proapoptotic Bcl-2 proteins that cause the release of cytochrome c from mitochondria ([Figure 16-2](#)). In cells dying by intrinsic mechanisms, cytochrome c triggers the formation of a protein complex called an apoptosome. The apoptosome recruits and activates caspase 9. Irrespective of their origin, the activated caspases then initiate a cascade of “executioner caspases” that degrades cytoplasmic and skeletal proteins, activates endonucleases, and results in cell

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death. Apoptotic cells characteristically break their DNA into many low-molecular-weight fragments. This fragmentation may be responsible for the characteristic chromatin condensation. In apoptotic cells dying by either pathway, the nuclear chromatin condenses against the nuclear membrane ([Figure 16-3](#)). Affected cells shrink and detach from the surrounding cells. Eventually nuclear break-up and cytoplasmic budding form cell fragments called apoptotic bodies ([Figure 16-4](#)).

As cells die, their cell membrane also “flips” so that the lipid phosphatidylserine is exposed on their surface. This lipid binds to receptors on macrophages and dendritic cells and triggers phagocytosis of the dying cell. It also triggers the release of antiinflammatory cytokines such as transforming growth factor- β (TGF- β) while inhibiting the release of proinflammatory cytokines such as TNF- α . When dendritic cells phagocytose apoptotic cells, they process the protein molecules from these cells and present them as antigen-MHC complexes on their surface. However, they do not express co-stimulatory molecules at this time. As a result, any T cells that recognize this antigen are not co-stimulated. They will be selectively turned off and tolerance will develop.

If cells are severely damaged as a result of trauma, toxicity, or microbial invasion, they will die and undergo necrosis. (Apoptosis is an active process; necrosis is a passive process.) Cells killed by necrosis trigger inflammation. Thus high mobility group box protein-1 from the cell nucleus is a potent inflammatory mediator. Likewise, when dendritic cells engulf necrotic cells, they not only process their proteins into MHC-antigen complexes, but they also express co-stimulatory molecules. T cells that recognize this antigen will therefore be turned on. Thus a cell killed by a virus will trigger significant inflammation and provoke a potent T cell response to the viral antigens.

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FIGURE 16-2 The two pathways by which apoptosis may be triggered. Both lead to caspase activation, DNA fragmentation, and cell death. The extrinsic pathway is activated by ligation of death receptors such as CD95 and formation of the death-inducing signaling complex (*DISC*). The intrinsic pathway is initiated by multiple damage signals, including injection of granzymes, and leads to the release of cytochrome C from mitochondria, the formation of an apoptosome, and activation of caspase 9.

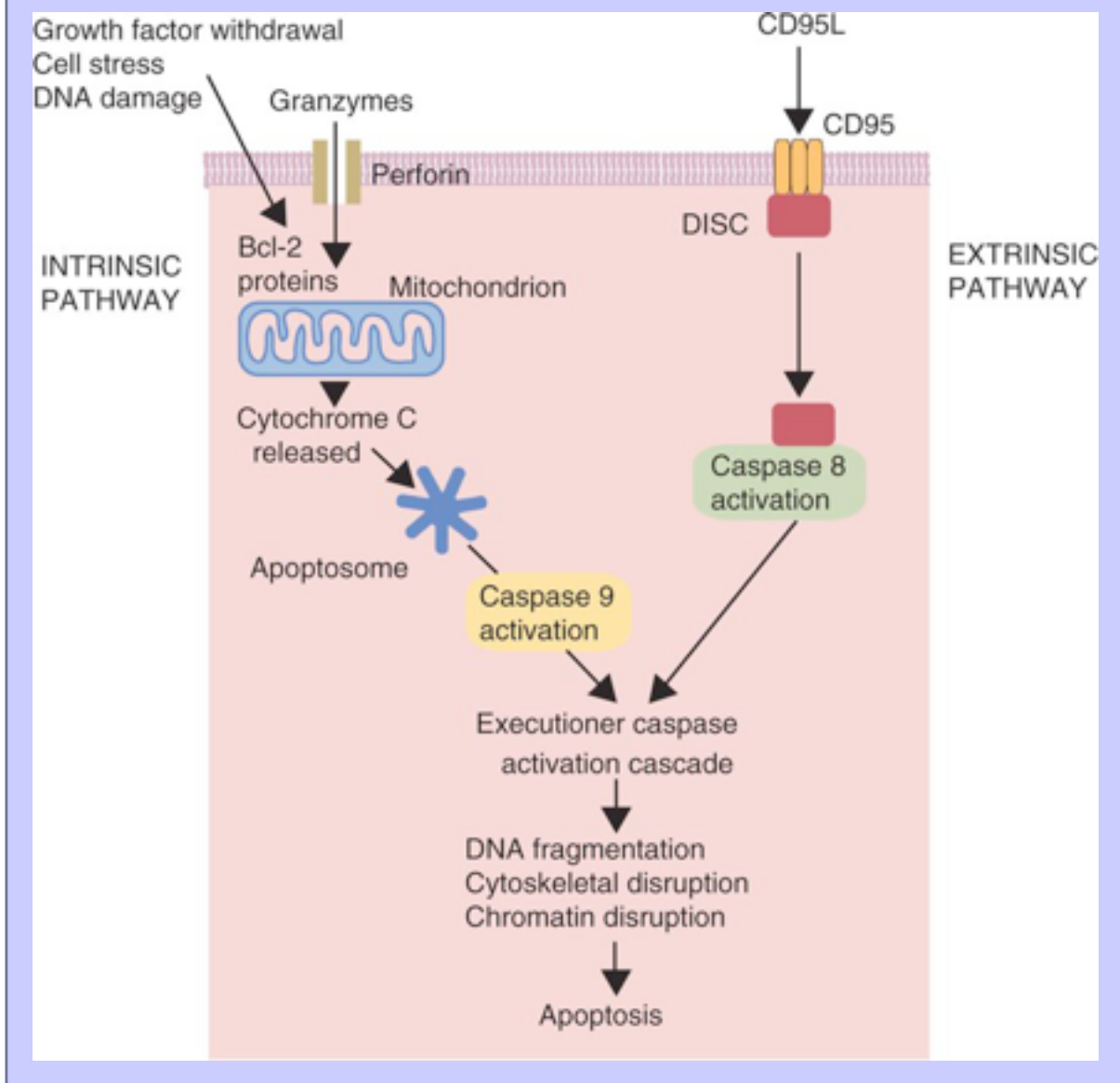


FIGURE 16-3 Major morphological features of cell death by apoptosis.

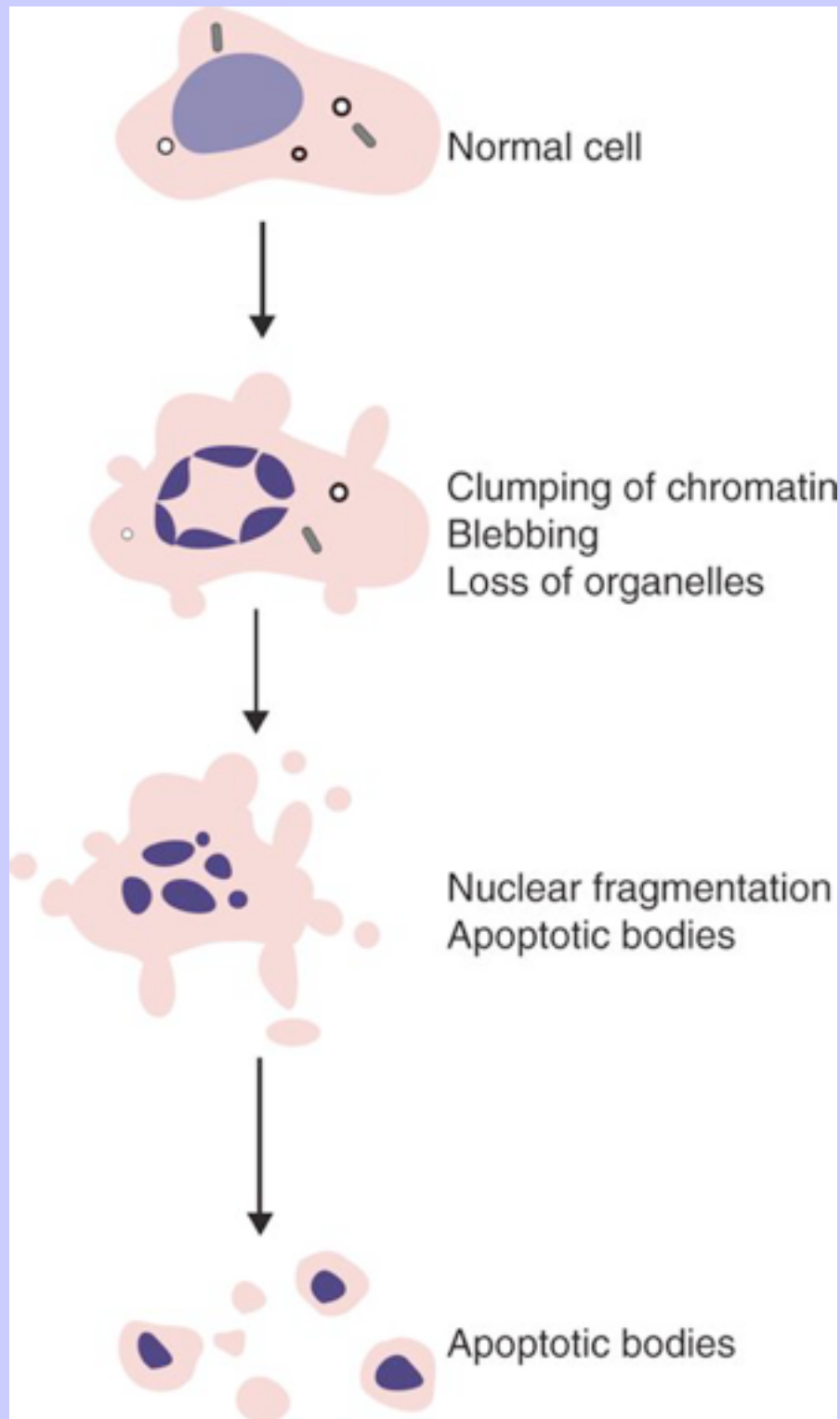
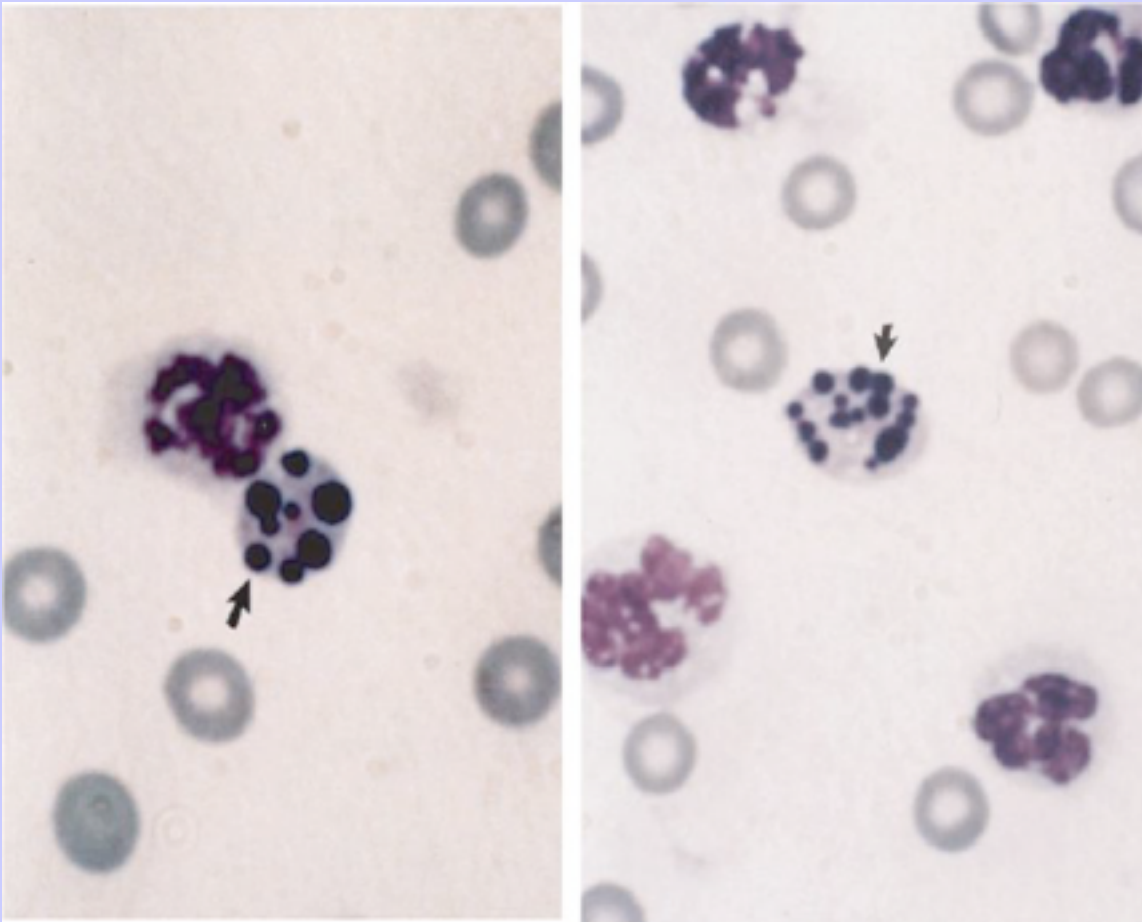


FIGURE 16-4 Two rat neutrophils showing nuclear condensation and fragmentation characteristic of apoptosis. (Courtesy Ms. K. Kennon.)



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16.4 CELL COOPERATION

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During a primary immune response, $CD8^+$ cytotoxic T cells cannot respond to infected cells by themselves alone. There are about 10^{13} nucleated cells in a human-sized body and possibly several hundred naïve T cells with receptors for any individual viral antigen. Clearly it would be almost impossible for these T cells to find individual virus-infected cells by themselves. Naïve cytotoxic T cells in fact remain within lymphoid organs and dendritic cells carry antigen to them. A subset of dendritic cells processes endogenous antigen, links it to their MHC class I molecules, and carries it to the lymphoid organs, where it is presented to $CD8^+$ T cells. These $CD8^+$ cells must also be co-stimulated by $CD4^+$ Th1 cells. Co-stimulation is only effective if both the $CD8^+$ and $CD4^+$ T cells recognize antigen on the same antigen-presenting cell. Thus a helper T cell first interacts with the antigen-presenting dendritic cell in the normal way through CD40 and CD154. Immature dendritic cells express low levels of MHC and co-stimulatory molecules and are thus poor T cell stimulators. T helper cells, however, activate the dendritic cells,

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upregulate their expression of MHC, and stimulate the production of IL-12 and the T cell chemotactic chemokine CCL22. Only when it is fully activated can a dendritic cell successfully trigger a cytotoxic T cell response.

The activated dendritic cells present peptides on MHC class I molecules to CD8⁺ T cells. They can do this readily if the dendritic cells are themselves infected. However, they can also present peptides from nonreplicating organisms or from dying infected cells. Thus by processing dying cells, dendritic cells activate T cells against endogenous antigens. These cytotoxic T cells do not require CD40/CD154 co-stimulation. The cytotoxic T cells require three key signals. The first is IL-12 from activated dendritic cells. The second signal comes from the antigen/MHC class I complex on an abnormal cell. The third signal comes from interleukin-2 (IL-2) and interferon- γ (IFN- γ) secreted by Th1 cells. Once all signals are received, the CD8 T cells can respond.

Different levels of stimulation trigger different responses in CD8 T cells. Thus T cell cytotoxicity is induced by a much lower threshold than cytokine synthesis. Likewise, although activated cytotoxic T cells can be stimulated by a brief exposure to antigen, naïve T cells have to be stimulated for several hours before responding. However, the required stimulation time may be shortened by increasing TCR occupancy or by providing additional co-stimulation. Once activated, cytotoxic T cell populations expand rapidly.

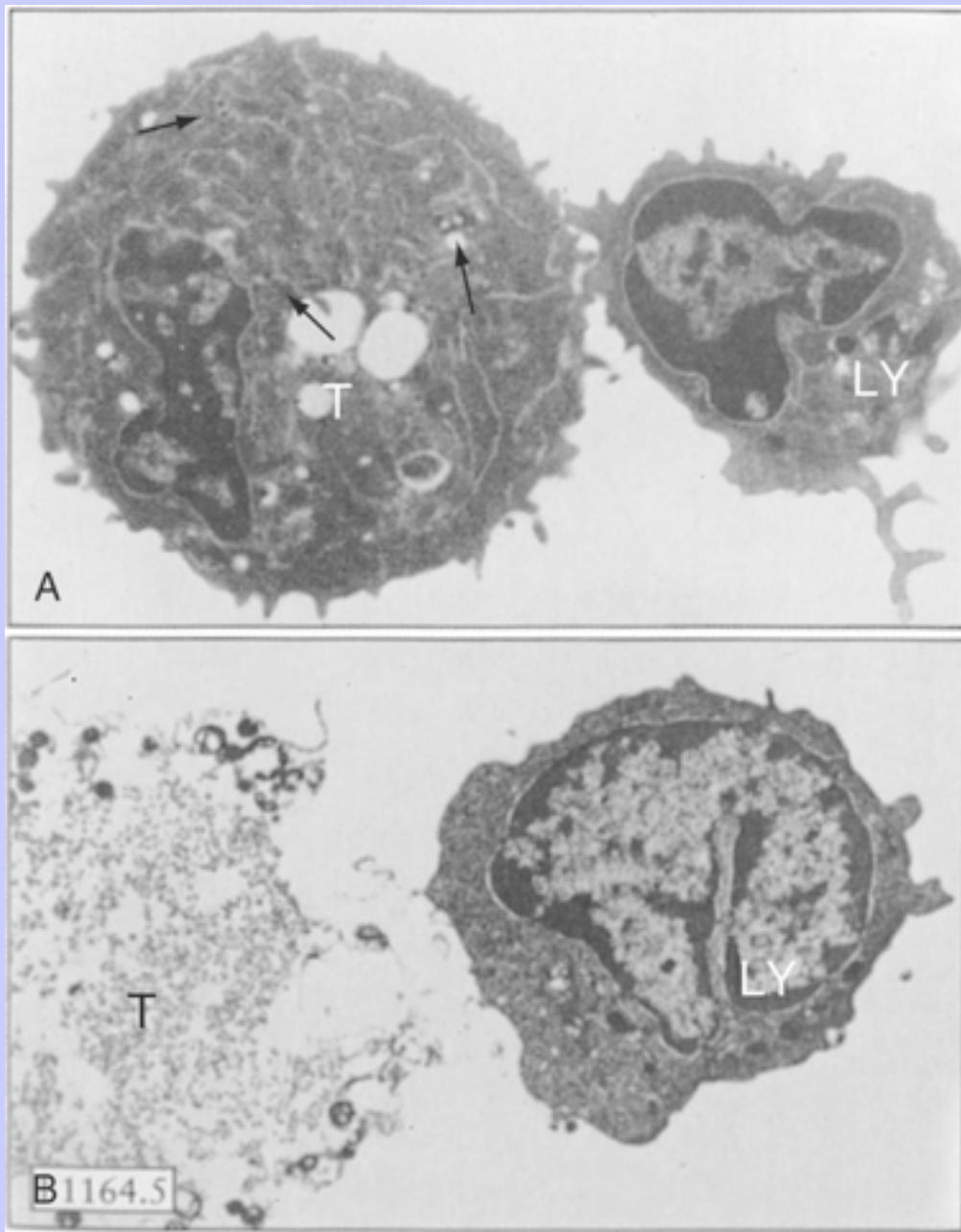
16.5

CYTOTOXIC T CELL RESPONSES

Once fully activated, CD8⁺ T cells leave the lymphoid organs and seek infected cells by themselves. When an activated CD8⁺ T cell recognizes that an MHC-antigen complex expressed on another cell and its TCR is occupied, the T cell will kill the target cell. Although most cells can only be triggered to undergo apoptosis by specific signals, cytotoxic T cells are able to induce apoptosis in any cell they recognize ([Figure 16-5](#)).

The density of peptide-MHC complexes on a target cell needed to stimulate T cell cytotoxicity is much lower than the density needed to stimulate cytokine production and clonal expansion. Thus a single peptide-MHC complex may be sufficient to trigger cytotoxicity, whereas 100-fold to 1000-fold more complexes are required to stimulate cytokine production and clonal expansion. Presumably cytotoxic T cells need to be highly sensitive to small amounts of viral peptides so that they can kill infected cells as soon as possible. These differences in signal thresholds are probably due to the structure of the immunological synapses formed.

FIGURE 16-5 Destruction of target cells by cytotoxic T cells. **A**, Conjugation between a peritoneal exudate lymphocyte (the small cell on the right) and a target cell. Note the lysosome-like bodies (LY) and the nuclear fragmentation of the target cell (T). **B**, A lymphocyte with the remains of a lysed target cell. (From Zagury D et al: *Eur J Immunol* 5:881, 1975.)



When T cells contact their target, an immunological synapse forms ([Figure 16-6](#)). The synapse has two “centers.” One center (central supramolecular activation cluster [cSMAC]) contains the TCR/CD8 complex. The other serves as the portal of entry for secreted cytotoxic molecules into the target cell. Both are surrounded by a pSMAC rich in adhesion molecules that forms a “gasket” preventing the accidental spill of cytotoxic molecules. Once a synapse has formed, cytotoxic T cell killing is highly efficient. They can kill a target cell within 2 to 10 minutes. Within seconds after contact between a T cell and its target, the organelles and the nucleus of the target cell show apoptotic changes. They are also serial killers that can disengage and move on to kill other targets within 5 to 6 minutes.

Cytotoxic T cells kill their targets through two pathways. One pathway involves the secretion of proteins called perforins and granzymes from secretory lysosomes (the perforin pathway) ([Figure 16-7](#)). This kills cells through intrinsic apoptotic mechanisms. The other pathway kills cells through the CD95 death receptor ([Figure 16-8](#)). The perforin pathway is used primarily to destroy virus-infected cells, whereas the CD95 pathway is used to kill unwanted T cells.

16.5.1 The Perforin Pathway

This pathway is used primarily to kill virus-infected cells. The killing process can be divided into three phases: adhesion, lethal hit, and cell death.

16.5.1.1 The Adhesion Phase

The CD8-TCR complex on a cytotoxic T cell binds to MHC I molecules on the target cell surface and an immunological synapse rapidly forms around the contact area. The TCRs and other signaling molecules cluster at one of the centers of the complex while they are surrounded by rings of adhesion molecules (see [Figure 16-6](#)). The CD8 molecules bind target cell MHC class I and enhance the binding between a T cell and its target. If the TCR has a very high affinity for the target, co-stimulation through CD8 molecules may not be necessary.

In addition to the signal from antigen-MHC-CD8 complexes, cytotoxic T cells need co-stimulatory signals. As with CD4 helper cells, CD8 cytotoxic cells are optimally activated only when their CD28 binds to CD86 on the target cell. The additional signal generated by CD28-CD86 binding permits cytotoxic T cells to kill target cells in the absence of IL-2 from Th1 cells. Tumors or virus-infected cells that express CD86 are much more sensitive to killing by cytotoxic T cells

FIGURE 16-6 Structure of the immunological synapse that forms between a cytotoxic T cell and its target. The outer ring of adhesive proteins forms an effective “gasket” that prevents leakage of cytotoxic molecules into tissue fluid. There are however two central supramolecular activation clusters (cSMACs). One is dedicated to signaling and so contains the T cell antigen receptor together with accessory molecules and co-stimulators. The other is dedicated to cytotoxic mechanisms. It is through this cSMAC that perforins, granzymes, and the Fas-FasL signals are transmitted.

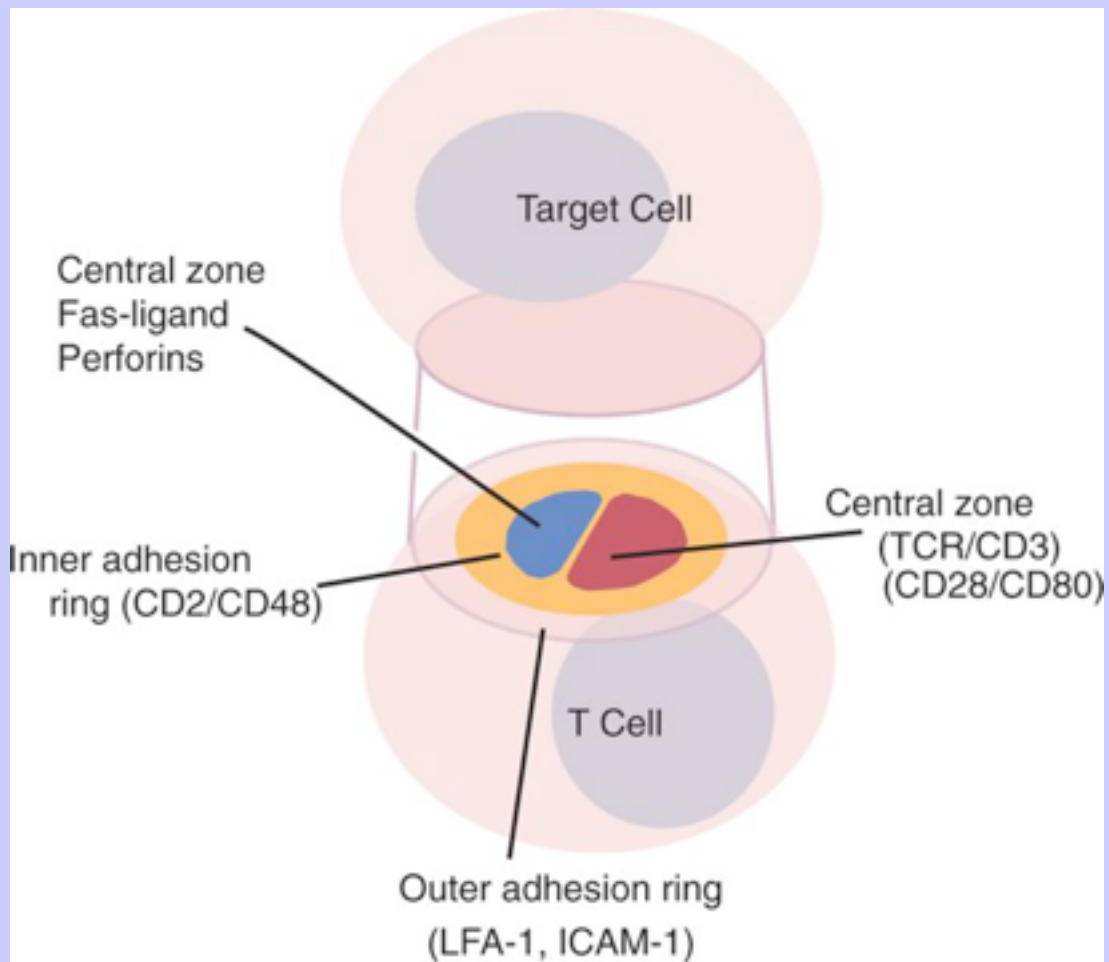
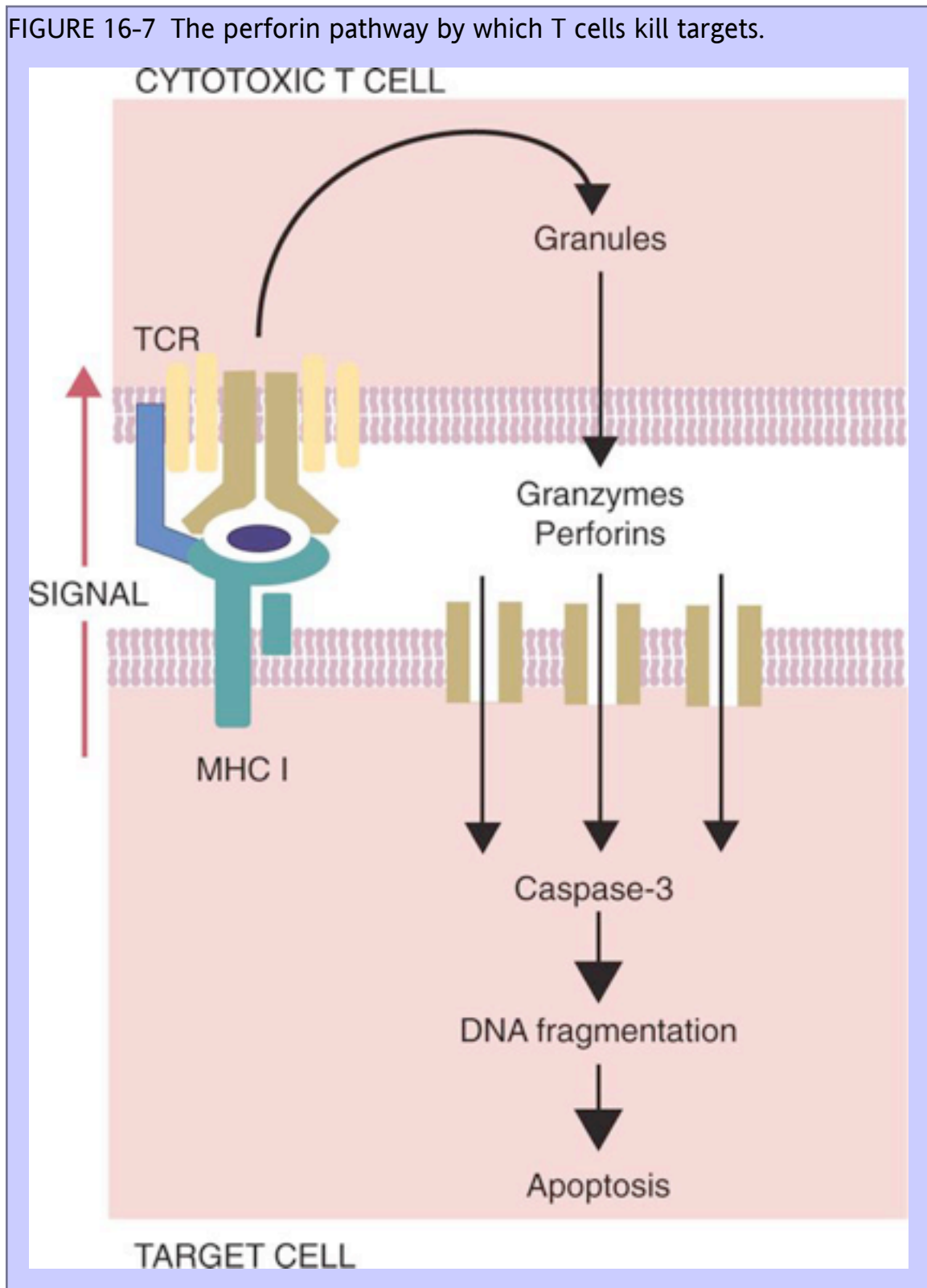


FIGURE 16-7 The perforin pathway by which T cells kill targets.



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FIGURE 16-8 The CD95 pathway of T cell-mediated cytotoxicity.

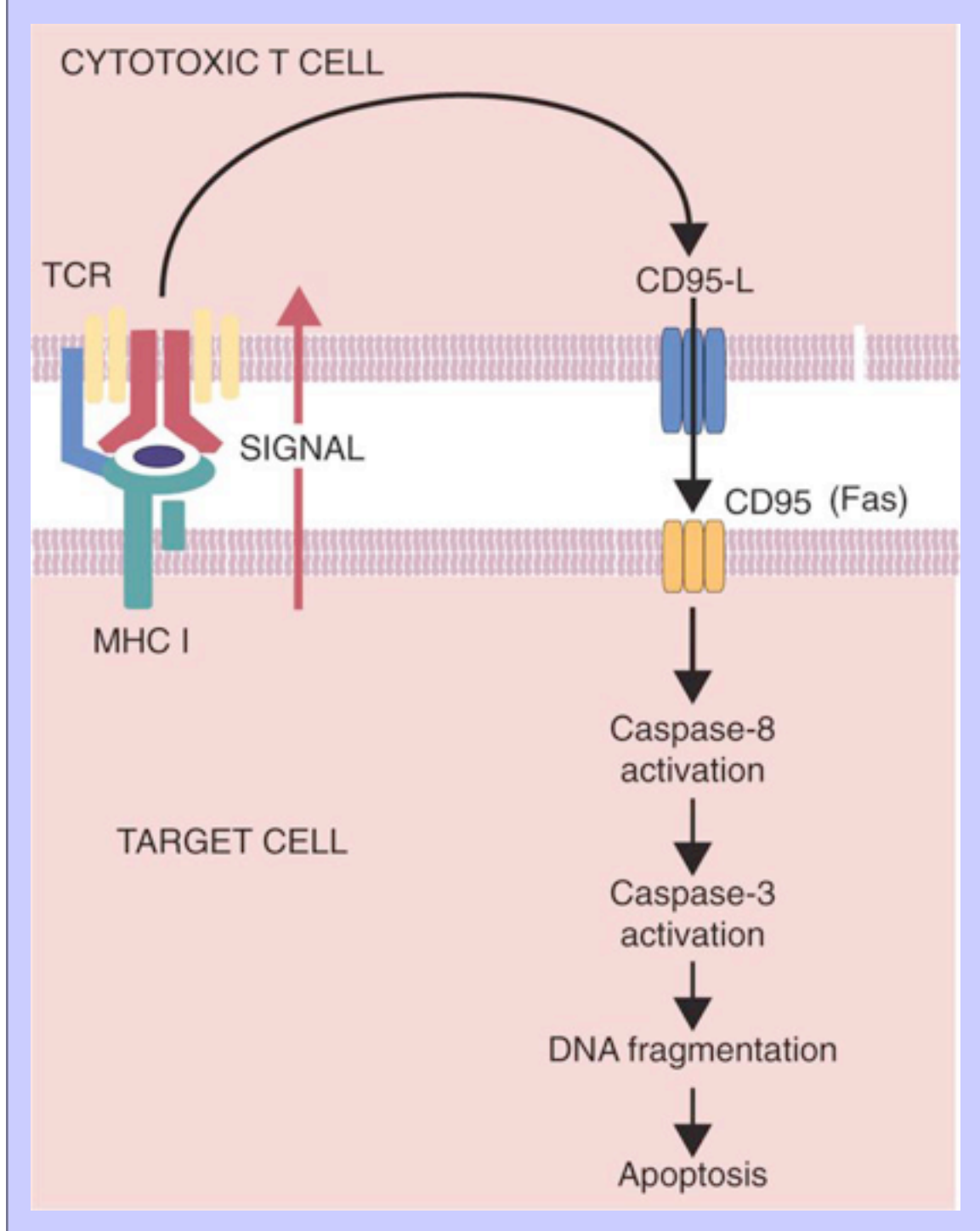
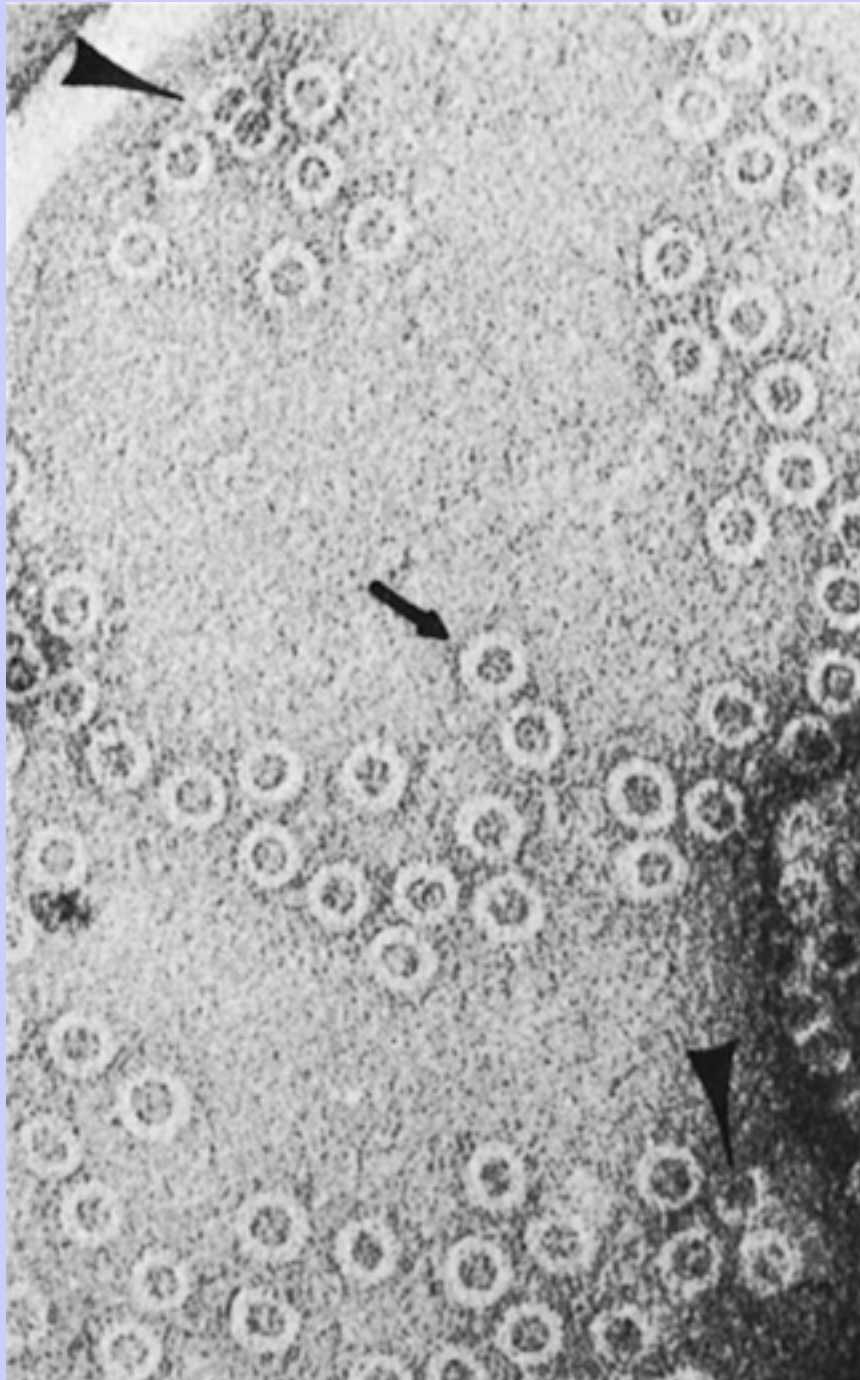


FIGURE 16-9 Perforins from human natural killer cells on the surface of a rabbit erythrocyte target. The arrowheads point to incomplete rings and double rings. (From Podack ER, Dennert G: *Nature* 301:44, 1983.)



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than target cells that do not express this molecule. Additional adhesion between cytotoxic T cells and their target is mediated by binding of T cell CD2 to target cell CD58 (in nonrodents) or CD48 (in rodents) and of CD11a/CD18 (LFA-1) and CD54 (ICAM-1).

16.5.1.2

The Lethal Hit

The first step occurs within a few minutes of binding to the target as the T cells orientate their microtubule organizing center, their Golgi complex, and their granules toward the target cell. The cytoplasmic granules migrate to the immunological synapse. Here they fuse with the T cell membrane in such a way that the toxic granule contents are directed straight into the target. Cytotoxic T cell granules contain several toxic molecules, the most important of which are perforins, granzymes, and granulysin. Cytotoxic T cells release the contents of these granules within the central secretory region of the immunological synapse.

Perforins are membrane-perturbing glycoproteins produced by cytotoxic T cells and natural killer (NK) cells. Perforins can insert themselves into the target cell membrane, but their precise mechanism of action is unclear.

In high concentrations in the presence of Ca^{++} , T cell perforins polymerize to form tubular transmembrane channels ([Figure 16-9](#)). Between 12 and 18 monomers aggregate to form a membrane attack complex that forms large (16 nm) lesions in target cell membranes. The perforins are related to and act in a similar manner to C9, the molecule that forms the complement membrane attack complex. While the central pore of the polyperforin may permit granzymes to enter target cells, killing also occurs at low perforin concentrations. It is believed that perforins release granzymes from target cell endosomes after endocytosis, thus permitting them to enter the target cell cytosol. Perforin activity in cytotoxic T cells is increased significantly by IL-2, IL-3, IL-4, and IL-6 and to a lesser degree by TNF- α and IFN- γ .

Granzymes are a family of at least 12 serine proteases found in T cells, where they account for about 90% of the total granule contents. Granzyme A is the most abundant and triggers apoptosis in target cells. It works synergistically with granzyme B. Granzyme A destroys histones and releases a nuclear DNase from repression, and this enzyme causes the DNA damage. Granzyme B then enters the target cell, either by injection through the central pore of the perforin complex or by endocytosis. It activates proapoptotic Bcl-2 proteins and so triggers the release of mitochondrial cytochrome c. As described above, the cytochrome c activates an apoptosome that in turn activates caspase 9 and the caspase cascade. Once activated, the caspase cascade leads to endonuclease activation, DNA fragmentation, and cell death.

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Granulysin is an antibacterial peptide found in the granules of both cytotoxic T cells and NK cells. A related molecule (Bo-lysin) is expressed in bovine T cells. It can kill target cells as well as a wide variety of extracellular pathogenic bacteria, fungi, and parasites. It shares homology with other proteins, called saposins, that attack lipid membranes. These are not pore-forming proteins but molecules that activate lipid-degrading enzymes such as sphingomyelinases. As a result, an increase in saposins increases the content of ceramide, which can induce apoptosis. The importance of granulysin lies in ensuring that lysed cells do not release viable bacteria. For example, cytotoxic T cells can control *Listeria monocytogenes* and *Mycobacterium tuberculosis* infections simply by killing infected cells. One problem with this process is that living bacteria released from the killed cells might infect healthy cells. To avoid this, the cytotoxic T cells release granulysin. This kills not only infected macrophages but also their intracellular bacteria.

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TNF- β , also called lymphotoxin- α (LT- α), is secreted by some cytotoxic T cells and has a similar mode of action to CD95L. TNF- β acts in one of two ways. Either it binds to LT- β in the T cell membrane to form a complex that kills target cells on contact or, alternatively, it binds to receptors on target cells and so triggers

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their apoptosis. Structural changes are seen by 2 to 3 hours, and by 16 hours more than 90% of target cells exposed to TNF- β are dead.

16.5.2 The Death Receptor Pathway

The second mechanism of T cell-mediated cytotoxicity involves binding between a T cell protein called CD95L (fas-ligand or CD178) and a target cell receptor called CD95 (fas). CD95L is expressed on activated CD8⁺ T cells and on NK cells. It binds to CD95 on target cells. When a T cell contacts its target, CD95L binds to CD95 and the CD95 trimerizes. This leads to the formation of a DISC that generates active caspase 8 and caspase 10 (see [Figure 16-2](#)). These enzymes in turn activate caspase 3 and trigger the apoptosis cascade. The CD95L-CD95 system regulates T cell survival. Unwanted surplus or self-reactive T cells are conveniently eliminated once they have served their functions. Thus when activated T cells have completed their task of killing their targets, they themselves undergo CD95-mediated apoptosis to downregulate the immune response.

In mice, *lpr* (lymphoproliferation) and *gld* (generalized lymphoproliferative disease) are loss-of-function mutations in the genes encoding CD95 and CD95L, respectively. Both mutations cause accumulation of activated T cells and accelerate autoimmune diseases. For example, *lpr* mice do not express CD95 on their thymocytes. As a result the thymocytes do not undergo apoptosis (negative selection) and are released into the secondary lymphoid organs. Here they proliferate excessively, resulting in a gross increase in the size of their lymphoid organs (lymphadenopathy). Because self-reactive cells are not destroyed, many of these cells respond to self-antigens and *lpr* mice develop an autoimmune disease similar to systemic lupus erythematosus (see [Chapter 33](#)).

16.6 CYTOTOXIC T CELL SUBSETS

As pointed out in [Chapter 12](#), there are two CD4⁺ T helper cell subsets, Th1 and Th2. Each subset is characterized by the mixture of cytokines it secretes. Subsets of CD8⁺ T cells have also been identified in rodents, where they are called Tc1 and Tc2. Tc1 cells secrete IL-2 and IFN- γ , whereas Tc2 cells secrete IL-4 and IL-5. A third subset, Tc0, has an unrestricted cytokine profile. Unlike helper cells that can differentiate readily into Th1 or Th2 cells, CD8⁺ T cells show a strong preference for the Tc1 phenotype. Differentiation into Tc2 requires exposure to large amounts of IL-4. All three subsets are cytotoxic.

Another way of classifying cytotoxic T cells is on the basis of CD8 expression. Thus in mice there are CD8⁺ cytotoxic T cells that kill *M. tuberculosis*-infected macrophages using granulysin and so destroy the mycobacteria. Mice also have a population of double-negative cells (CD4⁻CD8⁻) that kill targets through the CD95 pathway but do not inhibit mycobacterial growth.

In humans and mice, a large proportion of γ/δ T cells can recognize mycobacteria. *M. tuberculosis*-activated γ/δ T cells express IL-2 receptor, secrete IL-2, and can kill cells infected with this organism.

16.7 OTHER MECHANISMS OF CELLULAR CYTOTOXICITY

T cell-mediated cytotoxicity is not the only way by which the cells of the immune system can destroy abnormal cells ([Table 16-1](#); [Figure 16-10](#)). For example, cells that possess the antibody receptors Fc γ RI or Fc γ RII may bind target cells or bacteria through specific antibodies and then kill them. These cytotoxic cells may include monocytes, eosinophils, neutrophils, B cells, and NK cells (see [Chapter 30](#)). The mechanism of this antibody-dependent cell-

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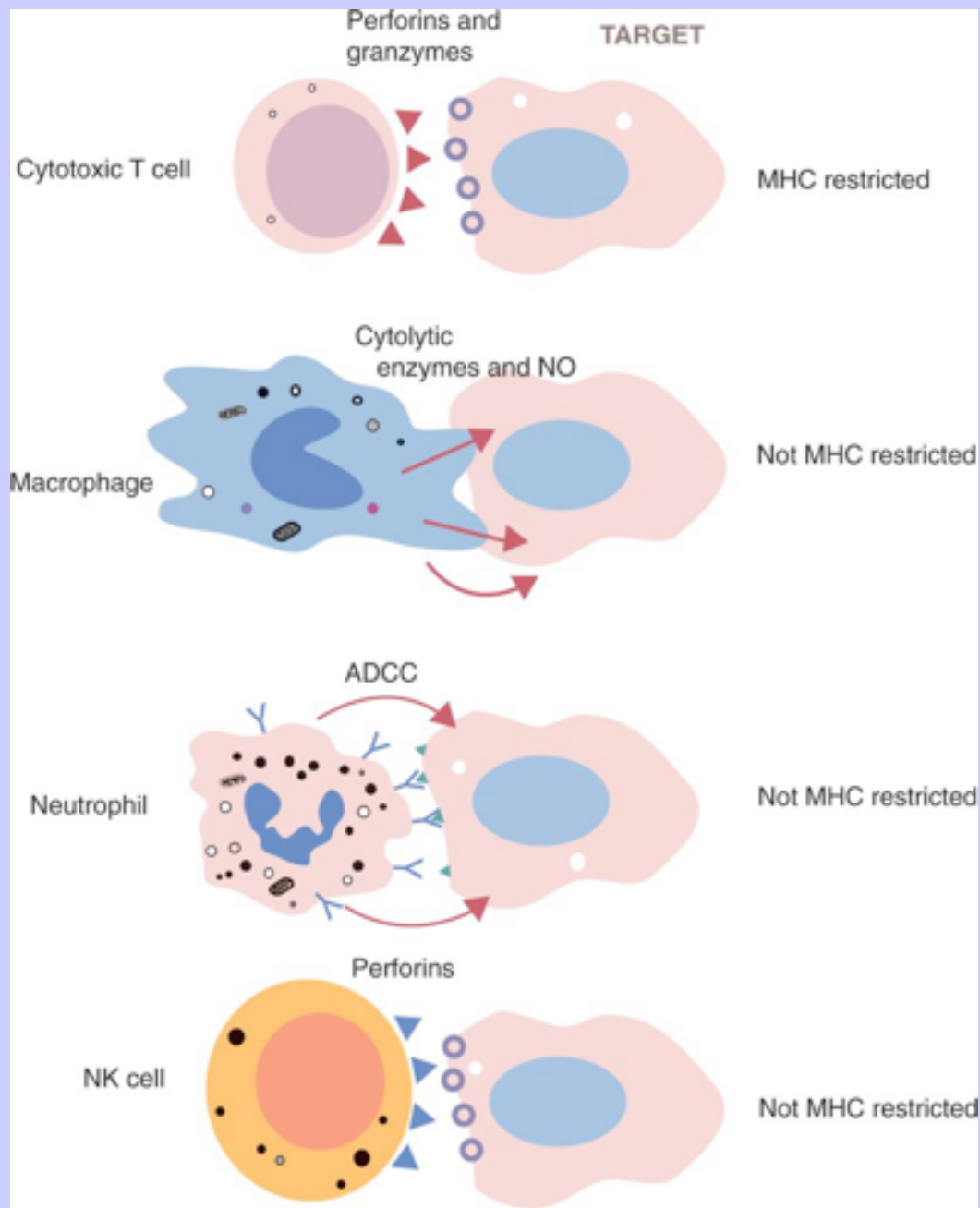
mediated cytotoxicity (ADCC) is unclear. However, neutrophils and eosinophils probably release oxidants. ADCC is slower and less efficient than direct T cell-mediated cytotoxicity, taking from 6 to 18 hours to occur.

Whether a macrophage participates in ADCC depends on its expression of Fc receptors and its

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FIGURE 16-10 Major pathways by which the cells of the immune system can kill nucleated target cells. These targets would normally be tumor cells or virus-infected cells.



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degree of activation. Macrophage-activating cytokines such as IFN- γ or granulocyte-macrophage colony-stimulating factor promote ADCC. Macrophages may also destroy target cells in an antibody-independent process. For example, when they ingest bacteria or parasites, macrophages release nitric oxide, proteases, and TNF- α . The nitric oxide will kill nearby bacteria and cells, whereas the TNF- α is cytotoxic for some tumor cells.

Table 16-1 A Comparison of the Three Major Mechanisms of Cell-Mediated Cytotoxicity

Cytotoxic Cells	Time (hr)	Mechanism	MHC Restricted	Antigen Specific
NK cells	24	NK-mediated cytotoxicity	No	No
Normal lymphocytes or macrophages with Fc γ RIII with specific antibody	6-18	ADCC activity	No	Yes
Primed T cells	<1	T cell mediated cytotoxicity	Yes	Yes

16.8 MACROPHAGE ACTIVATION

Although killing of infected cells by apoptosis is an important defense mechanism, there are occasions where such an extreme measure is not needed. It may be sufficient simply to activate macrophages so that these cells can effectively destroy invaders. For example, bacteria such as *L. monocytogenes*, *M. tuberculosis*, and *Brucella abortus* and protozoa such as

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FIGURE 16-11 Normal macrophages are killed by growing intracellular bacteria. Interferon- γ (IFN- γ) and interleukin-2 released by Th1 cells can activate macrophages and so enable them to kill otherwise resistant intracellular bacteria.

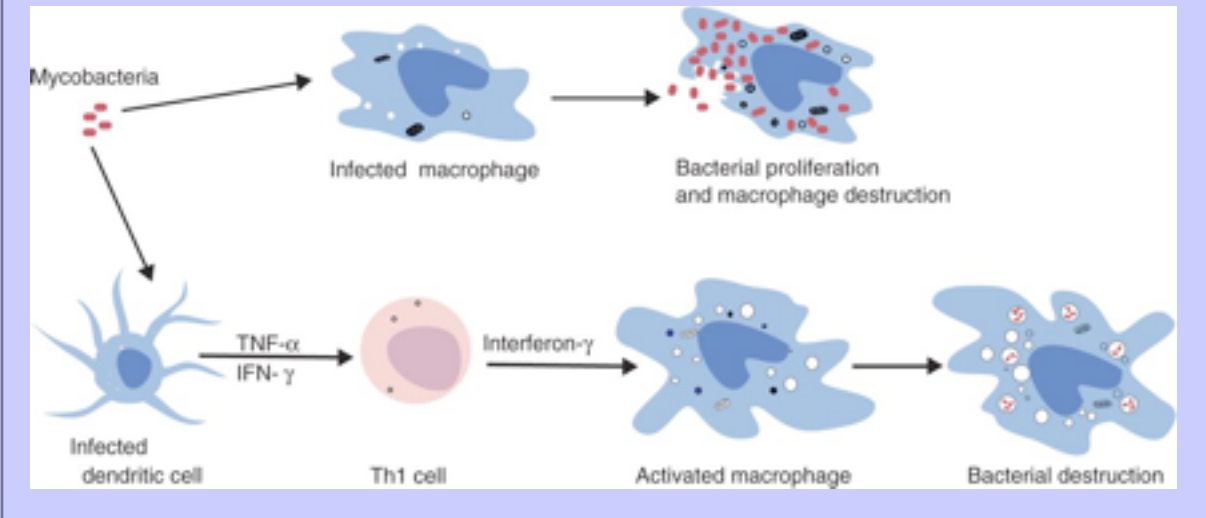
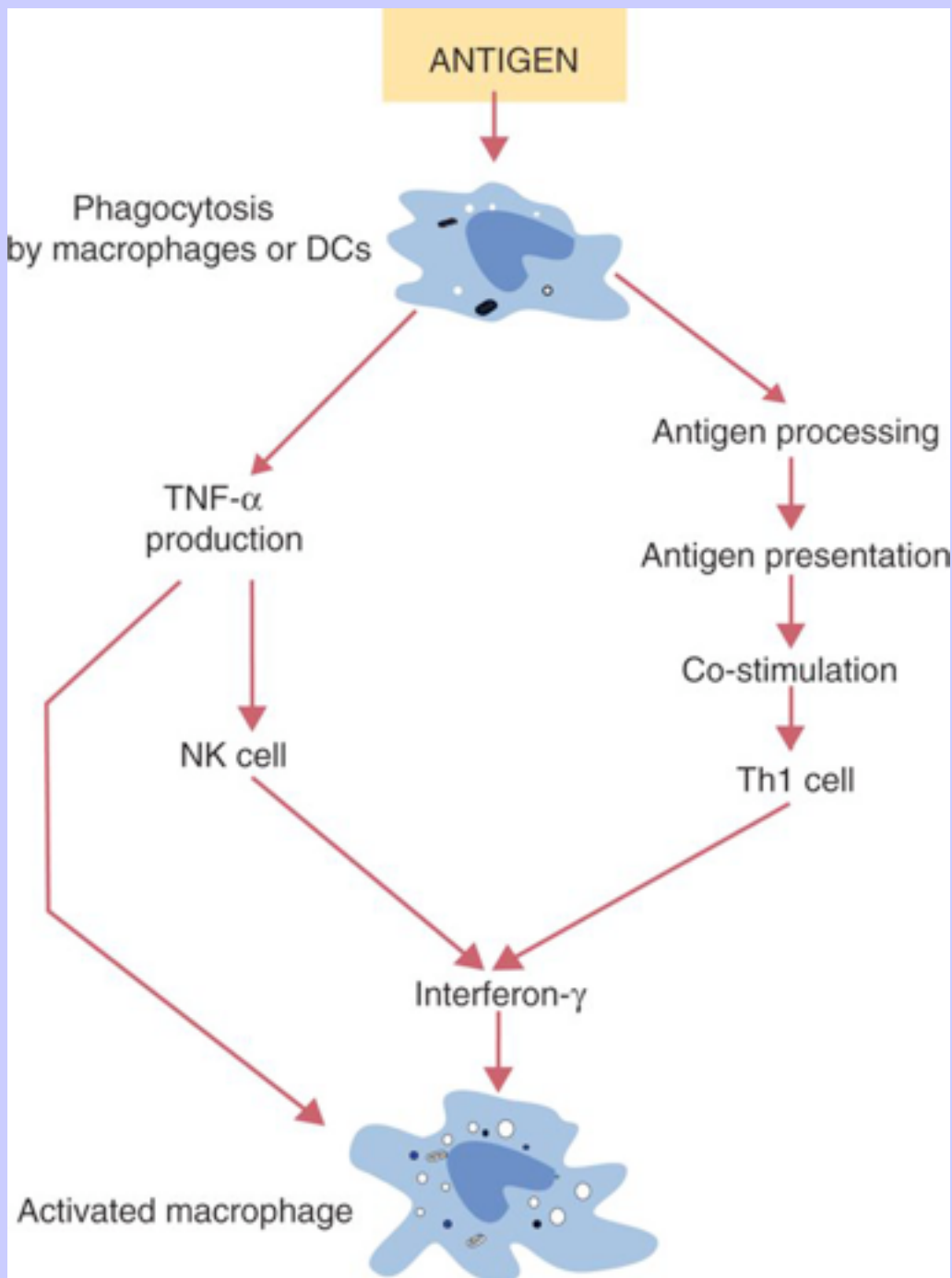


FIGURE 16-12 The two pathways by which macrophages can be activated. One involves interferon- γ (IFN- γ) production by natural killer cells and is thus an innate pathway. The other is mediated by IFN- γ from Th1 cells and so is an acquired response.



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Toxoplasma gondii survive and multiply inside normal macrophages. Antibodies are therefore ineffective against these organisms. Protection against this type of infection develops as a result of macrophage activation ([Figure 16-11](#)).

Activated macrophages are functionally polarized. Classically activated, or M1, macrophages are proinflammatory effector cells. Alternatively activated, or M2, macrophages have antiinflammatory effects and play a role in tolerance induction and in resolving inflammation.

16.8.1

Classical Macrophage Activation

M1 cells can be activated through several pathways. They can be activated through an innate pathway involving stimulation of toll-like receptors (TLRs), or they can be activated by IFN- γ and signals generated by Th1 cells. In general macrophages are maximally activated by both signals. A priming signal comes from IFN- γ . The second signal may come from TNF- α or, indirectly, from inducers of TNF- α production through TLRs ([Figure 16-12](#)). Thus the innate pathway is triggered when macrophages encounter microbial pathogen-associated molecular patterns such as mycobacterial glycolipids or lipoproteins through their TLRs. These receptors upregulate the production of TNF- α . The TNF- α then stimulates NK cells to secrete IFN- γ , which binds to its receptor on macrophages and so initiates activation.

In the T cell-mediated pathway, processed antigen triggers Th1 cells to secrete IFN- γ . The IFN- γ primes macrophages as described above. The necessary TNF- α comes from microbial invasion, or the second signal may be generated by interaction between CD40 on the macrophage and CD154 on a T cell. It is likely that the innate pathway works in the early stages of an infection, whereas the T cell-dependent mechanisms come into operation later.

Activated macrophages show major changes in their secretory profile. M1 cells activated by IFN- γ and

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FIGURE 16-13 M1 macrophage activation occurs through two linked signaling pathways. Microbial pathogen-associated molecular patterns acting through toll-like receptors (*TLR*) and nuclear factor kappa-B (*NF-κB*) leads to enhanced production of interleukin-12 (*IL-12*) and tumor necrosis factor- α (*TNF-α*). The second mechanism acts through the interferon- γ (*IFN-γ*) receptor and the Janus kinase/signal transducer and activator of transcription (*JAK/STAT*) pathway.

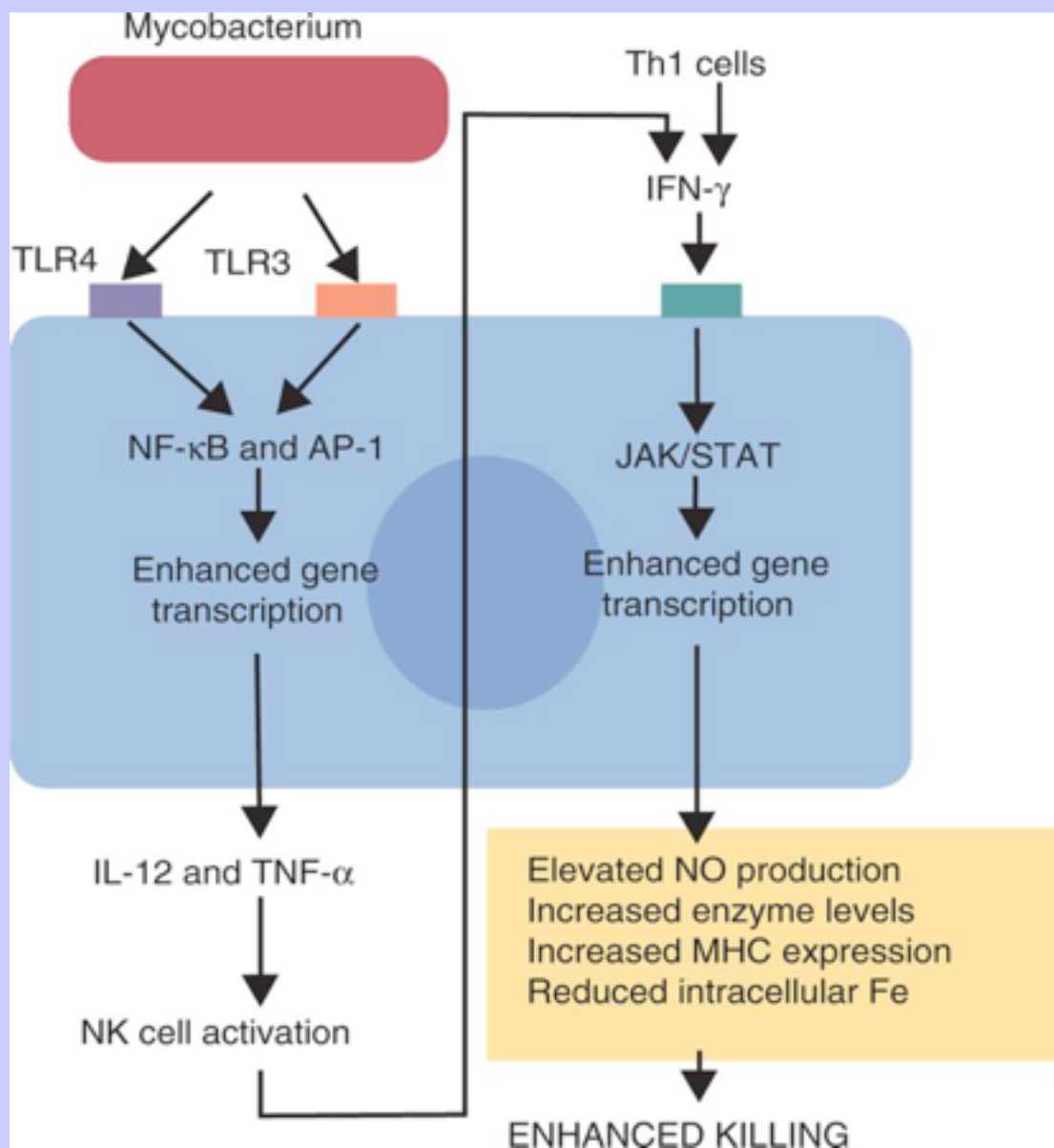
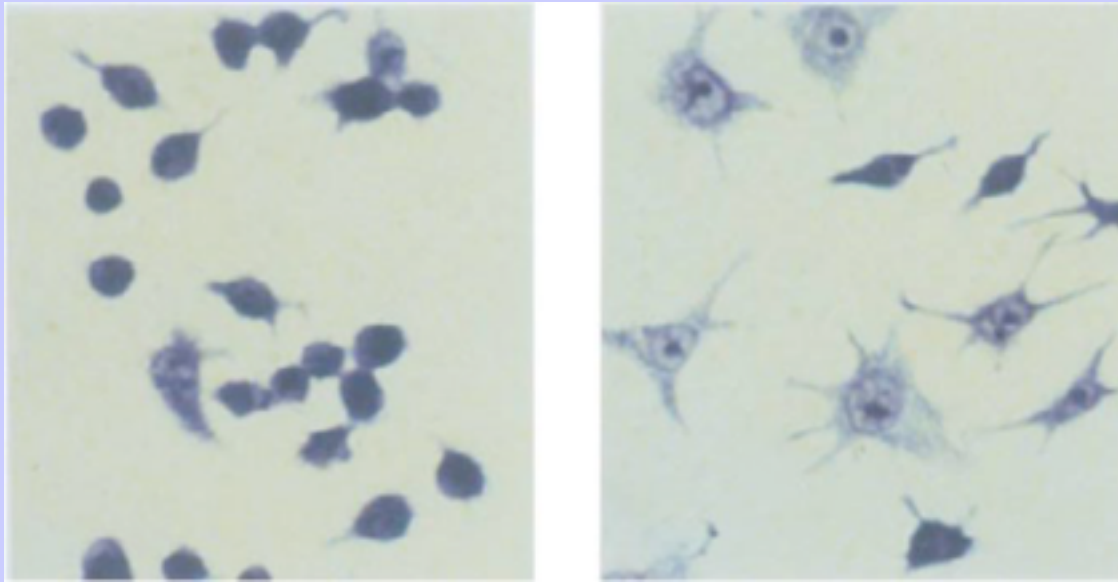


FIGURE 16-14 Stained cultures of mouse macrophages grown under identical conditions: *Left*, Normal unstimulated macrophages. *Right*, Macrophages activated by exposure to interferon- γ and acemannan. Note the cytoplasmic spreading of the activated cells. These cells secrete large quantities of cytokines and nitric oxide. Original magnification $\times 400$. (Courtesy Dr. Linna Zhang.)



TNF- α secrete large amounts of IL-12 and IL-23 but low amounts of IL-10. As a result they induce a strong Th1 response. Macrophage-derived nitric oxide also has an effect on T cells ([Figure 16-13](#)). By activating guanylate cyclase, it causes increased expression of the IL-12 receptor but has no effect on the IL-4 receptor. This also results in enhanced Th1 activity. Activated macrophages secrete proteases, which activate complement components. They secrete interferons, as well as thromboplastin, prostaglandins, fibronectins, plasminogen activator, and the complement components C2 and B. They express increased quantities of MHC class II molecules on their surface and so have an enhanced ability to process antigen.

M1 macrophages are enlarged and show increased membrane activity (especially ruffling), increased formation of pseudopodia, and increased pinocytosis (uptake of fluid droplets) ([Figure 16-14](#)). They move more rapidly in response to chemotactic stimuli. They contain increased amounts of lysosomal enzymes and respiratory burst metabolites, and they are more avidly phagocytic than normal cells. They produce greatly increased amounts of nitric oxide synthase 2. As a result, they can kill intracellular organisms or tumor cells by generating high levels of nitric oxide. The nitric oxide can destroy nearby tumor cells and intracellular bacteria such as *L. monocytogenes* ([Figure 16-15](#)). IFN- γ -activated macrophages can also inhibit the growth of intracellular bacteria such as *Legionella pneumophila* by limiting the availability of iron. They do this by downregulating their transferrin receptors (CD71) so that less transferrin is endocytosed and by reducing the concentration of intracellular ferritin, the major iron storage protein in macrophages. All these changes reduce the ability of the cells to support microbial growth.

On occasion, macrophage activation may result in a different type of polarized response whereby the cells differ in receptor expression, cytotoxic function, and cytokine production ([Figure 16-16](#)). For example, when activated through their FcγR or under the influence of Th2 cytokines such as IL-4, IL-10, and IL-13, macrophages become M2 cells. These M2 cells are functionally immunosuppressive and antiinflammatory. They work towards the resolution of inflammation and wound repair. They also serve a protective function in some parasitic diseases by walling off parasites such as schistosomes. Instead of producing nitric oxide, they use arginase I to produce ornithine. They secrete low amounts of IL-1α, IL-12, IL-23, and caspase I but large quantities of IL-10 and IL-1RA. As a result, they tend to promote Th2 responses. This important regulatory process is discussed in [Chapter 17](#).

TGF-β secreted by M2 cells stimulates extracellular matrix (ECM) production by nearby fibroblasts. M2 cells secrete the matrix component fibronectin. They secrete transglutaminase, which promotes ECM cross-linking, and osteopontin, which promotes cell-binding to the ECM. Arginase I is involved in proline and polyamine synthesis. Proline is involved in ECM construction, whereas polyamines are involved in cell proliferation. M2 cells secrete PDGF, IGF, and TGF-β, which promote cell proliferation. They secrete bFGF, TGF-α, and VEGF, which participate in angiogenesis. Thus the molecules secreted by M2 cells promote resolution of inflammation and wound repair and have antiinflammatory fibrotic, proliferative, and angiogenic properties.

The importance of these activation pathways can be seen in tuberculosis. Mycobacteria that enter the lungs are readily phagocytosed by alveolar macrophages that mount a respiratory burst and secrete proinflammatory cytokines. These cytokines act on NK cells triggering IFN-γ production and limited macrophage activation. This rapid innate response can slow mycobacterial growth significantly. Nevertheless these macrophages cannot destroy the bacteria by these mechanisms alone. After several days, recruitment of T cells occurs. The T cells are stimulated by

FIGURE 16-15 The destruction of *Listeria monocytogenes* when mixed in vitro with cultures of normal macrophages and “activated” macrophages from *Listeria*-infected mice.

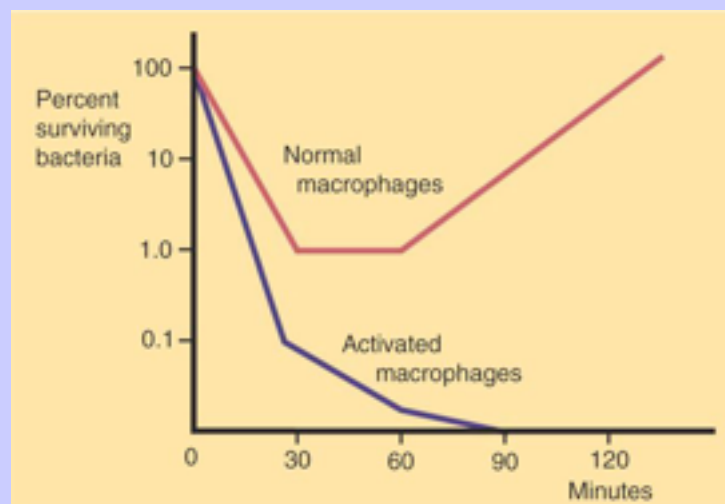
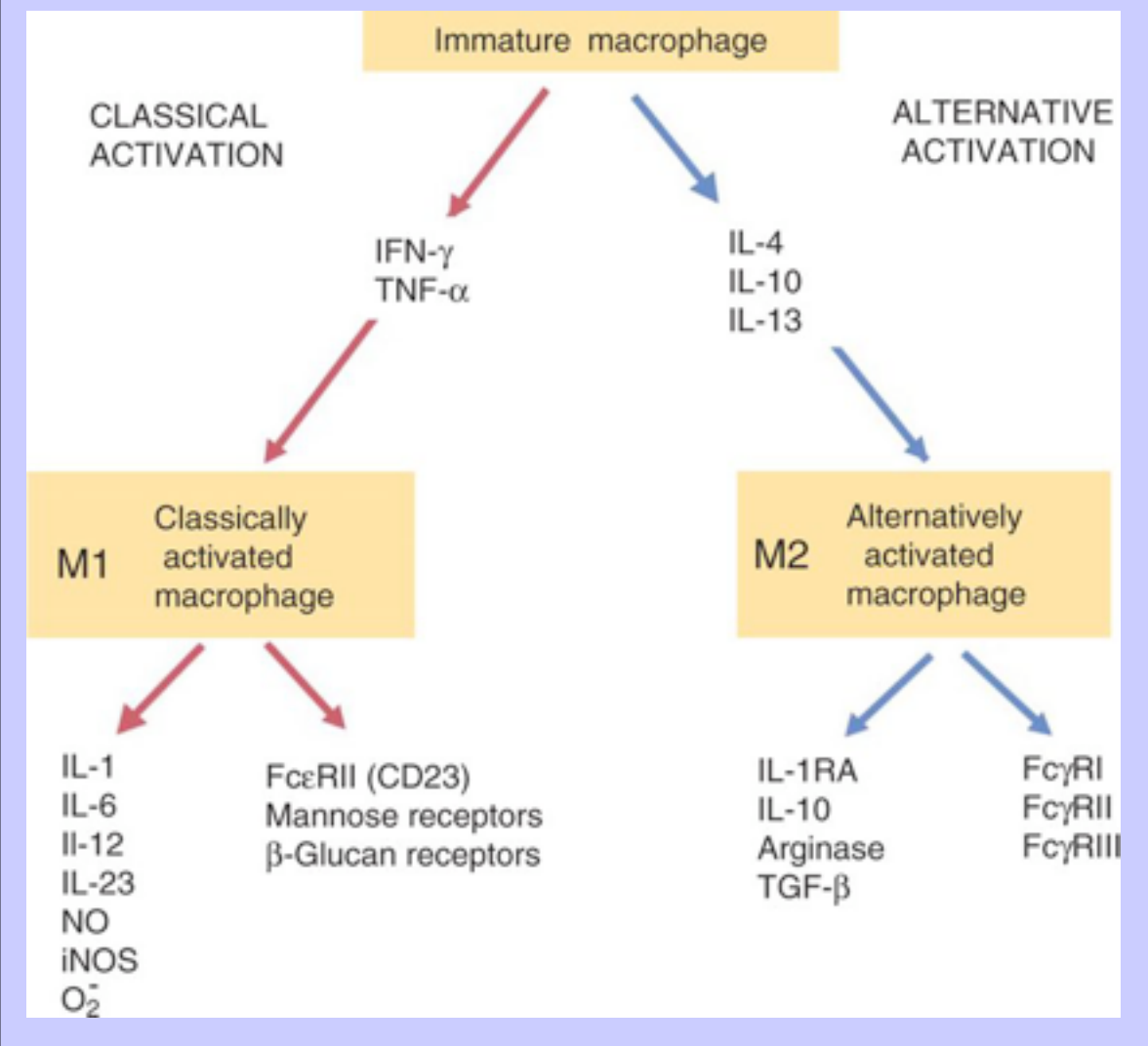


FIGURE 16-16 Depending on their cytokine exposure, macrophages may be classically activated (M1 cells) or become alternatively activated (M2 cells). M2 cells have a major regulatory role and are critical to granuloma formation and wound healing.



mycobacteria-infected dendritic cells secreting IL-12, TNF- α , and IFN- α . As a result, responding Th1 cells are stimulated to secrete IFN- γ and fully activate the macrophages about 10 days after onset of infection ([Table 16-2](#)). In most individuals, this level of activation is sufficient to control the infection.

Table 16-2 Effects of Cytokines on Macrophage Function

Cytokine	Major Source	Effect
IL-2	Th1 cell	Activates
IFN- γ	Th1 cell, NK cell	Activates
IFN- α/β	Macrophages, T cells	Activates
TNF- α	Macrophages, Th1 cells	Activates
TNF- β	Th1 cells	Activates
GM-CSF	Many cell types	Activates
IL-4	Th2 cells	Suppresses
IL-10	Th2 cells, macrophages	Suppresses
IL-13	Th2 cells	Suppresses
TGF- β	T cells	Suppresses

Cytotoxic T cells can interact with infected macrophages. For example, cytotoxic T cells generated in cattle infected with *M. bovis* will specifically kill infected macrophages. This cytotoxicity is mediated by both WC1⁺ $\gamma\delta$ and CD8⁺ T cells. Presumably any *Mycobacteria* released are killed by granulysin.

16.8.3 Delayed Hypersensitivity Reactions

When certain antigens are injected into the skin of a sensitized animal, a slowly developing inflammatory response, taking many hours to develop, may occur at the injection site. This is a T cell-mediated response called delayed hypersensitivity. Delayed hypersensitivity reactions are classified as type IV hypersensitivity reactions (see [Chapter 28](#)). An important example of a delayed hypersensitivity reaction is the tuberculin response (the skin reaction that occurs following an intradermal injection of tuberculin).

16.9 EFFECTOR T CELL MEMORY

In contrast to the prolonged antibody response, the effector phase of T cell responses is relatively brief. Indeed, cytotoxicity is seen only in the presence of antigen. This is logical. Sustained cytotoxic activities or overproduction of cytokines can cause tissue damage.

Naïve T cells are long-lived resting cells that continuously recirculate between the bloodstream and lymphoid organs. Once they encounter antigen these cells respond rapidly and multiply fast in an effort to keep pace with the growth of invading pathogens. The number of responding cells may increase more than 1000-fold within a few days. They reach a peak 5 to 7 days after infection when pathogen-specific, cytotoxic T cells can comprise 50% to 70% of total CD8⁺ T cells. Once the infection has cleared, most of these cells are superfluous. Therefore the vast majority undergo apoptosis 1 to 2 weeks after infection. Elimination of excess cytotoxic T cells is a tightly controlled process involving the CD95L-CD95 system. (In the absence of either CD95 or CD95L, you will recall, mice develop lymphoid hyperplasia and autoimmunity.) The survivors of this stage become long-lived memory

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cells. In acute viral infections, memory T cells probably develop only when antigen is eliminated. In chronic or persistent infections, cytotoxic T cells probably persist.

The number of surviving memory cells is directly related to the intensity of the primary response. In general only 5% to 10% of the peak number of cytotoxic T cells produced survive to become memory T cells. Survival may be a function of duration of exposure to antigen. Cells exposed to antigen for prolonged periods may die whereas cells exposed only briefly may live. The observation that overwhelming viral infections can exhaust the T cell pool and impair memory is consistent with this idea.

Memory T cells can be distinguished from naïve T cells by their immunophenotype, by the mixture of cytokines they secrete, and by their behavior. For example, memory T cells are CD44⁺ and express high levels of IL-2R β , a receptor that binds both IL-2 and IL-15. They express increased amounts of adhesion molecules so they can bind more efficiently to antigen-presenting cells. They produce more IL-4 and IFN- γ and respond more strongly to stimulation of their TCR. Their enhanced responses may also be due to possession of higher-affinity IL-2 receptors. They continue to divide, very slowly, in the absence of antigen. This division requires cell-bound IL-15 and is inhibited by soluble IL-2. IL-15 is a unique cytokine that persists for very long periods attached to its receptor on T cells. It thus acts as a persistent source of IL-15 for the memory cell microenvironment and stimulates nearby cells by cell-cell contact. Thus the balance between IL-15 and IL-2 regulates the persistence of memory T cells. In the absence of IL-15 the memory cells undergo apoptosis. In humans the CD8⁺ memory cell half-life is 8 to 15 years.

16.10

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¹⁷ CHAPTER 17 Regulation of Acquired Immunity

^{17.1} KEY POINTS

- T cells must be made tolerant to self-antigens. This may be through central tolerance, where self-reactive T cells are killed. Alternatively it may be through peripheral tolerance, where these T cells are “turned-off” by inappropriate signaling.
- B cells are much harder to tolerize than T cells. They are generally regulated by peripheral mechanisms and by the absence of T help.
- Antigens stimulate immune responses, although very low or very high doses of antigen may cause tolerance.
- Antibodies tend to suppress antibody production through negative feedback mechanisms. This can prevent the successful vaccination of newborn animals as a result of maternal immunity.
- Immune responses may also be controlled by the activities of regulatory T cells.
- The immune system and the central nervous system are closely interconnected and influence each other.

The acquired immune system is a sophisticated defense system. It can recognize and respond to foreign invaders and can learn from the experience so that the body responds faster and more effectively when exposed to the invader a second time. However, there is a risk associated with this—the risk of damaging normal body components. One reason the acquired immune system is so complex is that considerable effort must be put into ensuring that it will attack only foreign or abnormal tissues and will ignore normal healthy tissues. As might be anticipated, many different mechanisms ensure that the chances of developing autoimmunity are minimized. In addition, immune responses must be regulated to ensure that they are appropriate with respect to both quality and quantity ([Figure 17-1](#)).

Since both T and B cells generate antigen-binding receptors at random, it is clear that the initial production of self-reactive cells cannot be prevented. An animal cannot control the amino acid sequences and hence the binding specificity of these receptors. As a result, between 20% and 50% of the T cell antigen

FIGURE 17-1 Some of the bad things that can happen if the immune system is not carefully regulated.

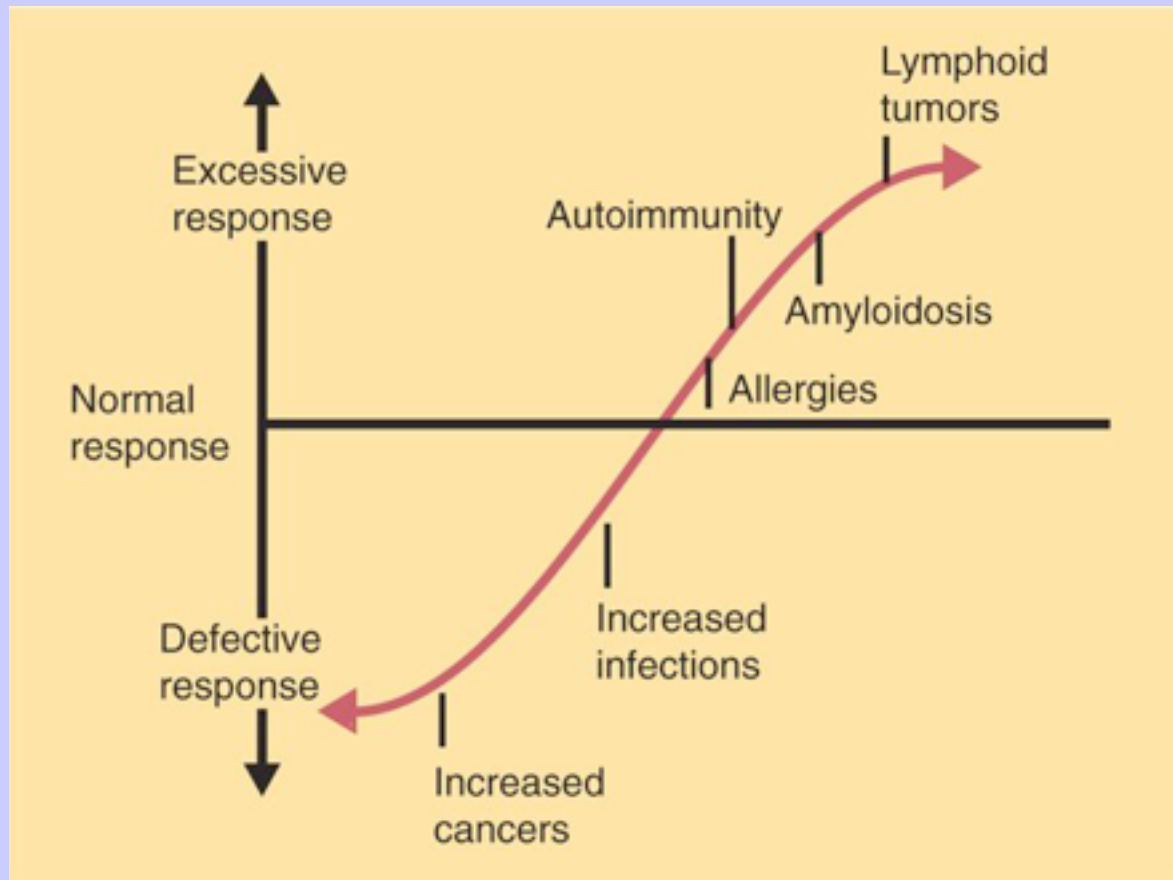
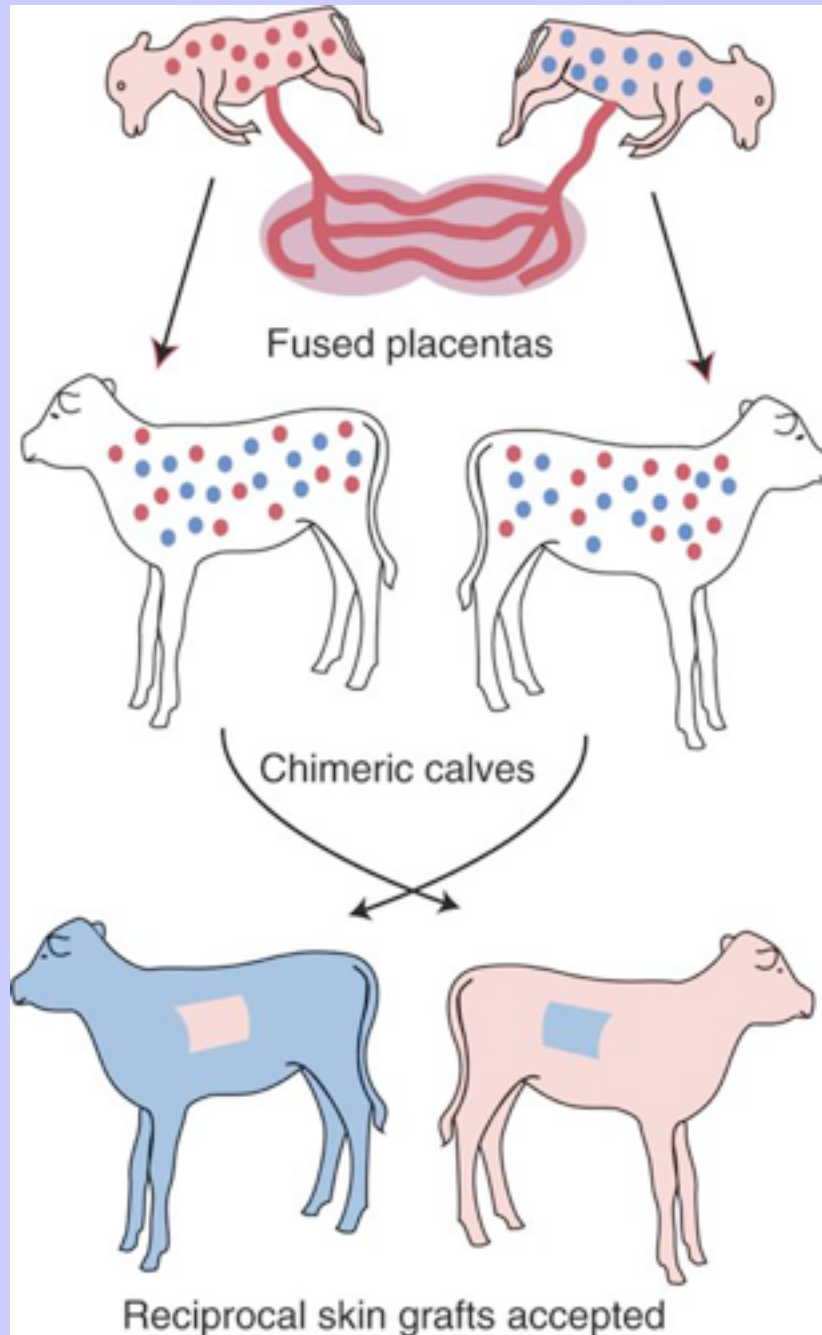


FIGURE 17-2 Fusion of the placentas of dizygotic twin calves results in the development of calf chimeras. Hematopoietic stem cells from each animal colonize the bone marrow of the other. Each chimera is tolerant to its twin's cells and so will accept a skin graft from its twin despite the genetic differences.



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receptors (TCRs) and B cell antigen receptors (BCRs) first generated may bind self-antigens. Self-tolerance therefore requires that those lymphocytes with in-appropriate receptors must either be destroyed or turned off.

17.2 TOLERANCE

Tolerance is the name given to the situation where the immune system will not respond to a specific antigen. Tolerance is primarily directed against self-antigens from normal tissues. In 1948 Burnet and Fenner recognized the need for self-tolerance and suggested that immature lymphocytes would become tolerant to an antigen if they first met it early in fetal life.

Support for this suggestion came from observations on chimeric calves. In 1945 Owen noted that when cows are carrying twin calves, blood vessels in the two placentas commonly fuse. As a result, the blood of the twins intermingles freely, and bone marrow stem cells from one animal colonize the other. Each calf is born with a mixture of blood cells, some of its own and some originating from its twin. In dizygotic (nonidentical) twins this is called a chimera. These “foreign” blood cells persist indefinitely because each chimeric calf is fully tolerant to the presence of its twin's cells ([Figure 17-2](#)). Burnet and Fenner suggested that this could only happen because each calf was exposed to the foreign cells early in fetal life during a period when lymphocytes become tolerant upon encountering antigens. Cells from an unrelated calf would be rejected normally if administered after birth.

Subsequent studies have shown that self-tolerance is of two types, central and peripheral. In central tolerance, immature self-reactive lymphocytes within the thymus, bursa, or bone marrow either die or alter their receptor specificity. In peripheral tolerance, mature lymphocytes that encounter self-antigens either die, are turned off (anergy), or are suppressed by regulatory T cells. Reconstituting lethally irradiated mice with T and B cells derived from normal or tolerant donors shows that tolerance can occur in both cell populations. However, their susceptibility to peripheral tolerance induction differs considerably. Thus T cells can be made tolerant rapidly and easily within 24 hours, and remain in that state for more than 100 days ([Figure 17-3](#)). In contrast, B cells develop tolerance in about 10 days and return to normal within 50 days.

17.3 T CELL TOLERANCE

17.3.1 Central T Cell Tolerance

17.3.1.1 Negative Selection

Tolerance will result if there are no functional T cells with receptors that can bind self-antigens ([Figure 17-4](#)). Although the body has available an enormous diversity of TCRs, far fewer receptors are actually used by mature T cells than might be anticipated. Several processes limit receptor diversity. First, the mechanisms used to generate TCR diversity inevitably result in the production of nonfunctional receptors. For example, two thirds of possible gene arrangements will be out of frame. Cells bearing these nonfunctional TCRs die by apoptosis. As T cells mature within the thymus, positive selection ensures that the cells that recognize self-major histocompatibility complex (MHC) molecules survive. At this point, however, the cells whose receptors bind too strongly to self-antigens are killed ([Figure 17-5](#)). The timing and extent of this killing depend on the affinity of the TCR for a self-antigen. T cells that bind self-antigens strongly are killed earlier and more completely than weakly binding cells. Thus the T cells that eventually leave the thymus have been purged of dangerous, self-reactive cells.

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The negative selection process is assisted by the presence of many different self-antigens in the thymus. Normally each tissue or cell type possesses its own tissue-specific antigens. Thus “skin antigens” are restricted to the skin, whereas “liver antigens” are restricted to the liver. However, the epithelial cells in the thymic medulla are unique in that they show “promiscuous” gene expression. These thymic epithelial cells contain a transcription regulator, called the autoimmune regulator, that promotes the expression of many different proteins once thought to be restricted to other tissues. Examples include insulin, thyroglobulin, and myelin basic protein. In this way, the thymic epithelial cells ensure that self-reactive T cells encounter many normal tissue antigens within the thymus and are therefore eliminated. In addition, some normal tissue antigens are taken up by macrophages and carried to the thymus. Self-reactive T cells that respond to these antigens are also eliminated. However, this raises another question: What about self-antigens that are not expressed in, or do not enter, the thymus? For example, antigens in the eye, testis, or brain are not processed in this way, and as a result central tolerance to these antigens may not develop.

When both positive and negative selection occur within the thymus, all the cells that can bind self-MHC molecules are positively selected, and then those that bind MHC molecules with very low or very high affinity are subsequently deleted. Thus the moderate-affinity clones survive and are able to recognize foreign antigens. An additional factor that probably determines whether a cell will live or die is the dose of antigen presented to cells within the thymus. Thus if the amount of a specific antigen is high (as one might anticipate for a self-antigen), multiple TCRs will be occupied on each thymocyte and this may trigger apoptosis. In contrast, if only low concentrations of an

FIGURE 17-3 The duration of tolerance in T cells and B cells. T cells are much more easily rendered tolerant than B cells. Once tolerant, they remain that way for much longer.

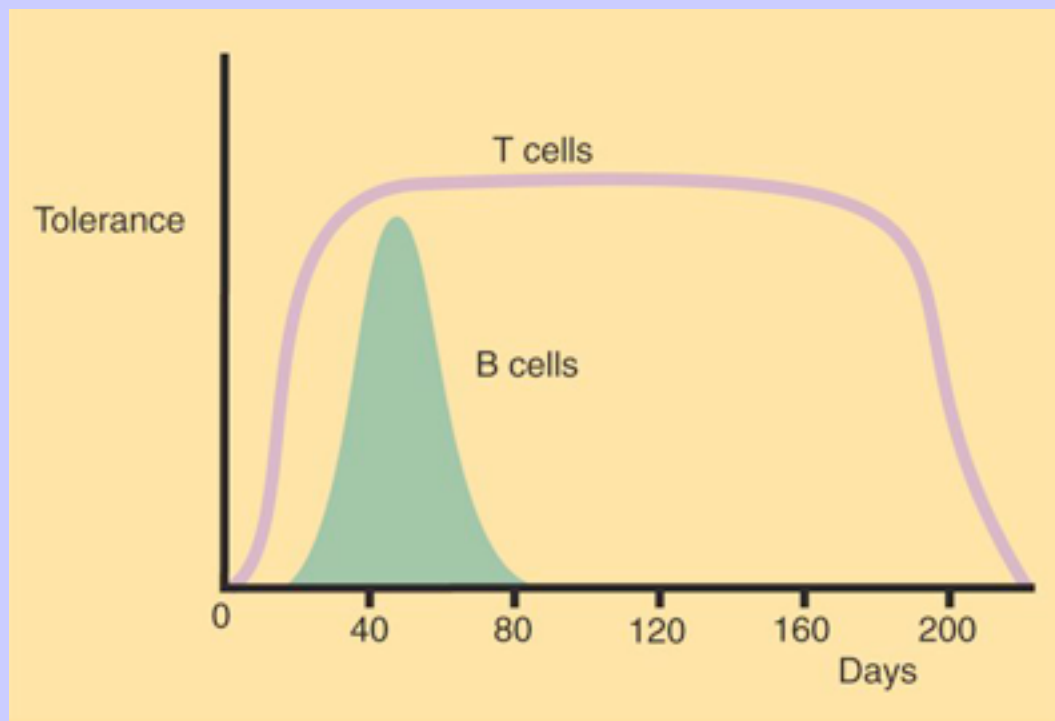
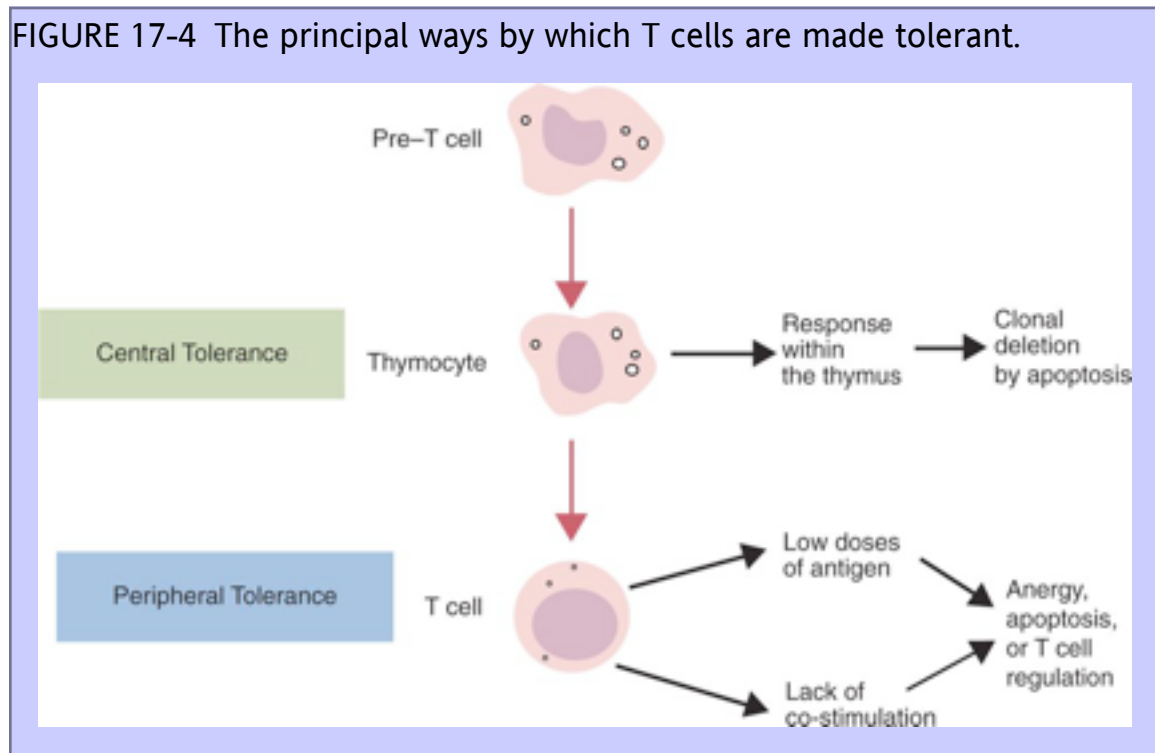


FIGURE 17-4 The principal ways by which T cells are made tolerant.



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FIGURE 17-5 How the thymus induces central T cell tolerance by negative selection. Surviving T cells are unreactive to autoantigens yet can still respond to foreign antigenic peptides in association with major histocompatibility complex (MHC) molecules as a result of positive selection.

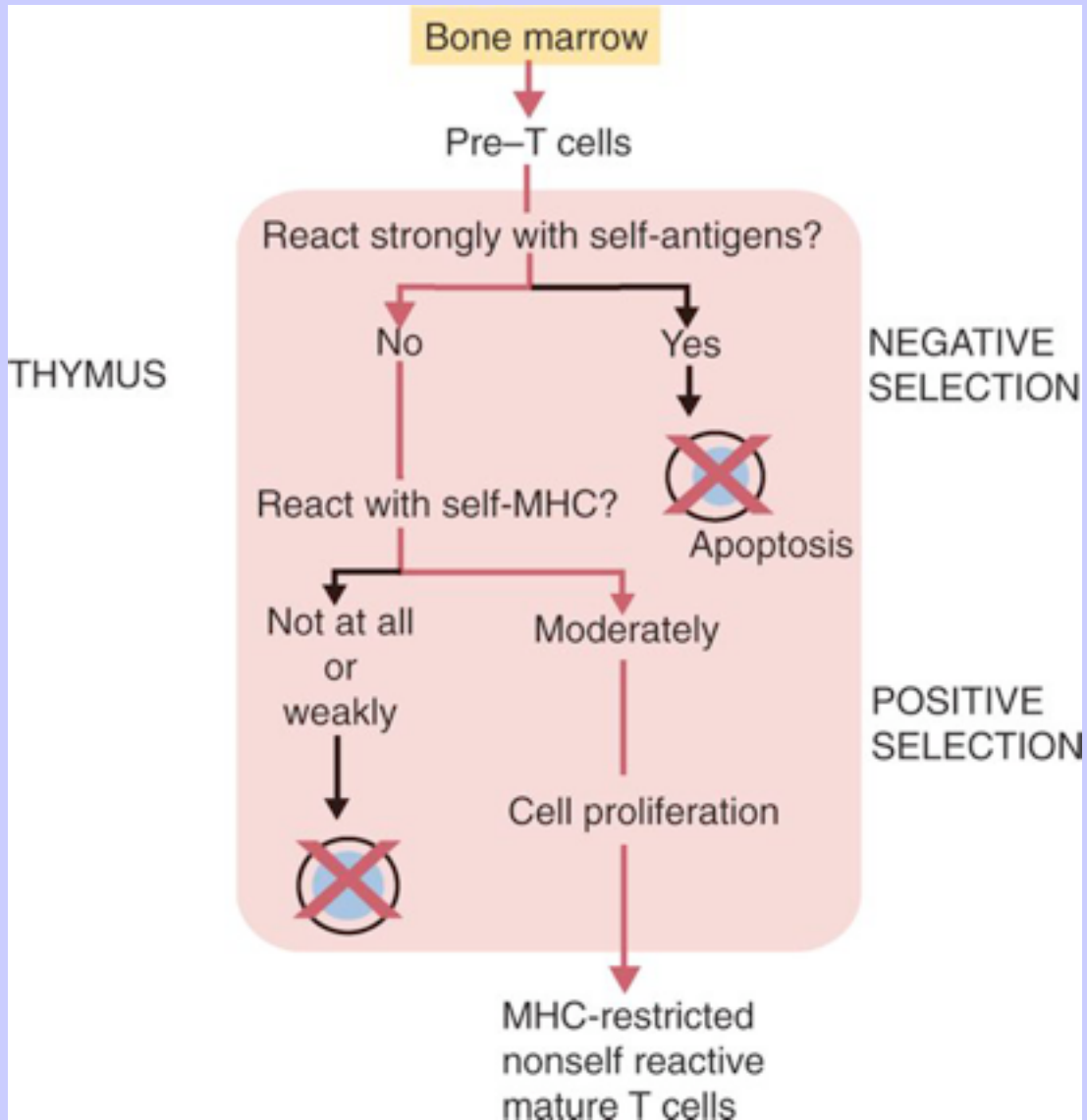
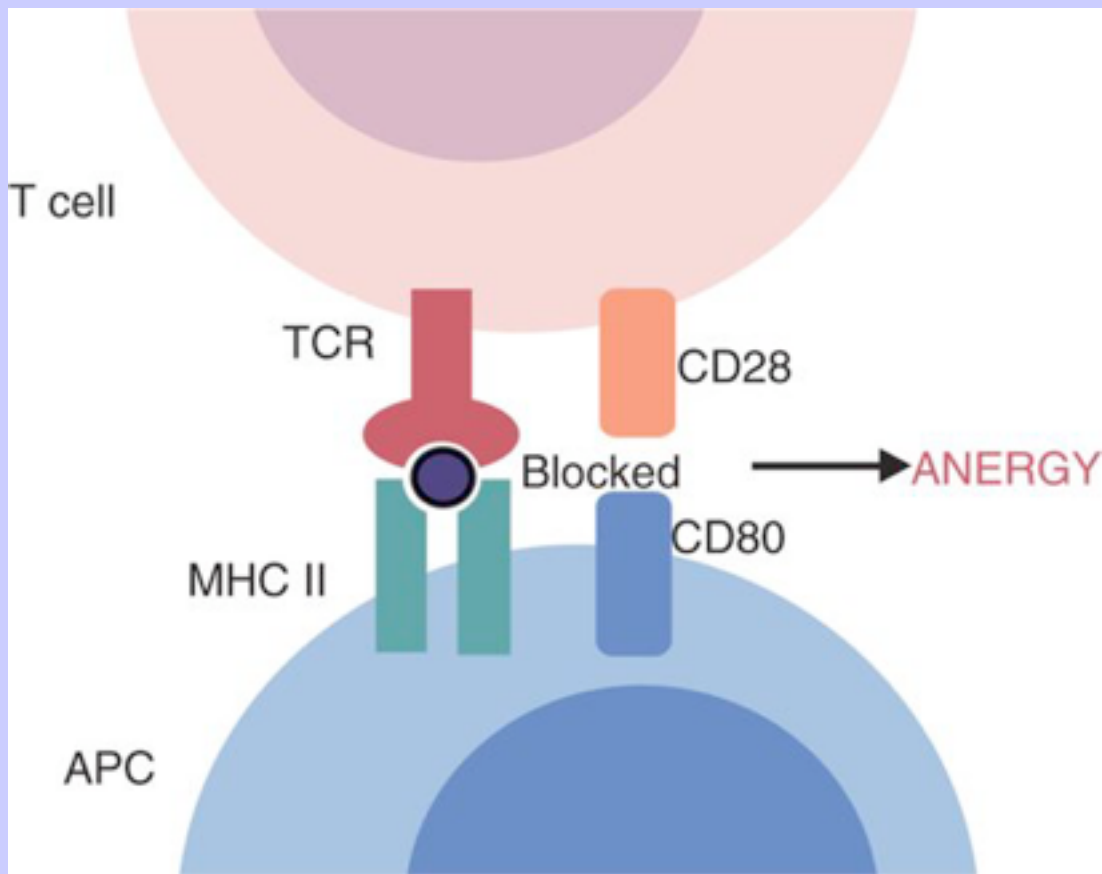


FIGURE 17-6 Peripheral tolerance through clonal anergy will develop if a T cell receptor is stimulated by antigen in the absence of simultaneous co-stimulation through the CD28/CD80 or CD28/CD86 pathway.



antigen are present, these will occupy only a few TCRs on each thymocyte. This level of signal may cause positive selection and thymocyte proliferation.

17.3.1.2

Receptor Editing

When the antigen receptors of a developing T cell bind to self-antigens, another strategy employed to prevent autoimmunity is receptor editing (see [Chapter 15](#)). In this process cell maturation stops but its recombinase activating genes remain active and V(D)J recombination continues. As a result, the TCR receptor a chain is replaced or diluted with another a chain. If a cell successfully edits its receptors, its maturation can proceed. Failure to do so will result in its apoptosis.

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17.3.2 Peripheral T Cell Tolerance

17.3.2.1 Clonal Anergy

Low-affinity self-reactive T cells may leave the thymus and must be suppressed by peripheral tolerance. One form of peripheral tolerance is clonal anergy. Clonal anergy—the prolonged, antigen-specific suppression of T cell function—depends on the signals delivered to a T cell. T cells require specific signals from several sources in order to respond to antigen. If these signals are insufficient or inappropriate, then T cells will not be activated and they become inactive. For example, protein solutions normally contain some aggregated molecules. These aggregated molecules are readily taken up and processed by dendritic cells and thus are highly immunogenic. If a solution of such a protein, such as bovine gamma globulin, is ultracentrifuged so that all the aggregates are removed, then the aggregate-free solution will induce anergy. It occurs because the T helper cells are exposed to free soluble antigens in the absence of the co-stimulatory signals normally provided by an antigen-presenting cell ([Figure 17-6](#)).

As pointed out in [Chapter 12](#), binding of an antigen to a TCR is by itself insufficient to trigger T cell responses. Indeed, occupation of the TCR by an antigen in the absence of co-stimulation causes tolerance. Binding to the TCR by an antigen activates the tyrosine kinases and phospholipase C of the T cell and raises its intracellular Ca^{2+} . This results in production of I κ B, which inhibits nuclear factor kappa-B (NF- κ B) and effectively turns the cell off. Only if interleukin-12 (IL-12) binds to its receptor *and* CD28 binds to CD80 will the production of the repressor be blocked and the cell activated. Tolerant Th1 cells produce about 1% to 3% of normal IL-2 levels and much less interferon- γ (IFN- γ). Once induced, their anergy can last for several weeks.

Very high doses of an antigen can induce a form of clonal anergy called immune paralysis ([Figure 17-7](#)). The high doses of the antigen probably bypass antigen-presenting cells, reach the T helper cell receptors directly, and in the absence of co-stimulation make the cells anergic.

17.4 B CELL TOLERANCE

Unlike the TCR repertoire, antibody diversity is generated in B cells in two phases. The first phase occurs by VDJ rearrangement or gene conversion in the primary lymphoid organs; the second phase involves random somatic mutation in secondary lymphoid organs. B cells therefore have several opportunities to generate receptors for self-antigens. It has been estimated that 55% to 75% of early immature B cells have self-reactive receptors, so suppression of these self-reactive B cells begins at an early stage in an animal's development.

Very immature B cells within the bone marrow can be made tolerant once they have successfully arranged their V-region genes and are committed to express complete immunoglobulin M (IgM) molecules on their surface. When these immature cells bind antigen strongly, the BCR transmits a signal that arrests cell development and triggers apoptosis. An immature B cell population can be rendered tolerant by one millionth of the dose of an antigen required to make mature B cells tolerant. B cells of young animals may also be unable to regenerate their cell surface immunoglobulins after synapse formation.

Immature B cells may also undergo receptor editing as described above and the developing B cell may develop a replacement light chain. If receptor editing fails to generate a non-self-reactive B cell, it will die.

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17.4.1 Peripheral B Cell Tolerance

Peripheral B cell tolerance can be induced by apoptosis, clonal anergy, clonal exhaustion, and blockage of BCRs.

Because BCRs undergo random somatic mutation within germinal centers, self-reactive B cells can still occur in peripheral lymphoid organs. These cells will not make autoantibodies if antigen-presenting cells and T helper cells are absent or if regulatory T cells are active ([Figure 17-8](#)). This is not, however, a foolproof method of preventing self-reactivity. In the absence of T cell help, B cells may receive a second signal through their TLRs from bacterial lipopolysaccharides, flagellins, or unmethylated CpG DNA. B cells may also be activated by the use of either cross-reacting epitopes or by a foreign carrier molecule stimulating nontolerant T helper cells (see [Chapter 31](#), [Figure 31-2](#)).

As with T cells, B cell anergy results if the B cells encounter antigens in the absence of co-stimulation. B cells are difficult to maintain in a tolerant state,

FIGURE 17-7 Capacity of different doses of antigen to induce peripheral tolerance. Both very low and very high doses can induce tolerance. Moderate doses, in contrast, induce an immune response.

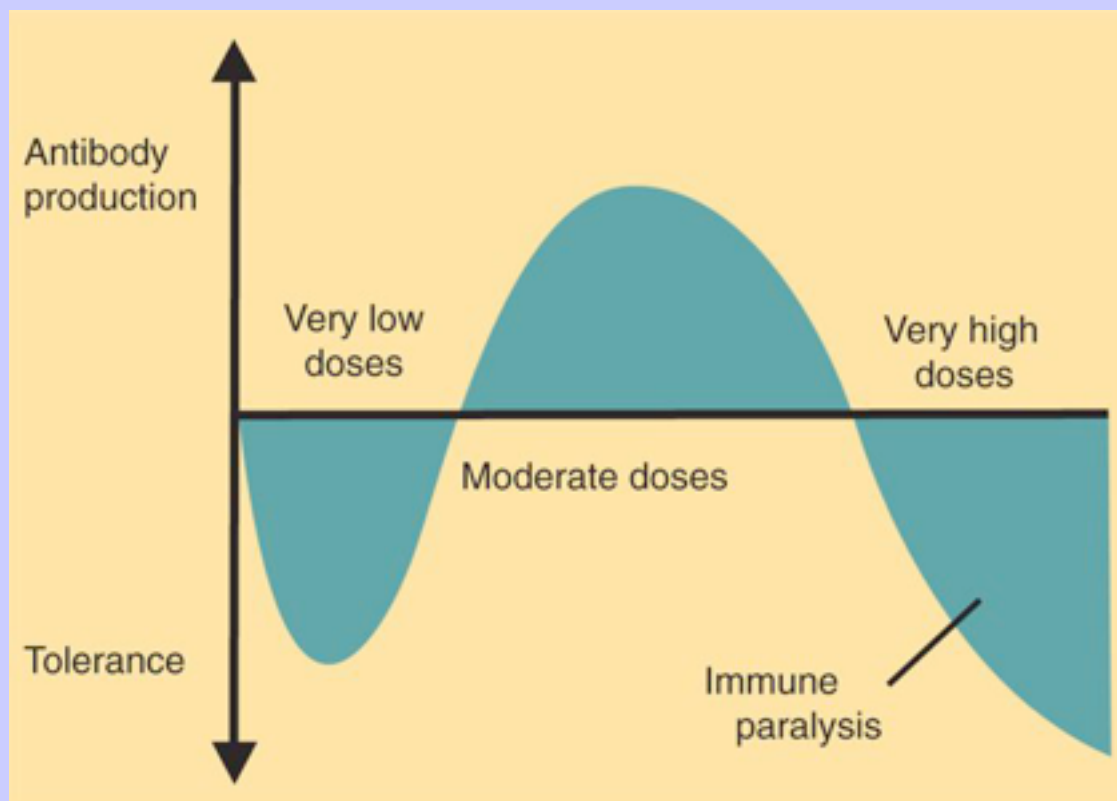
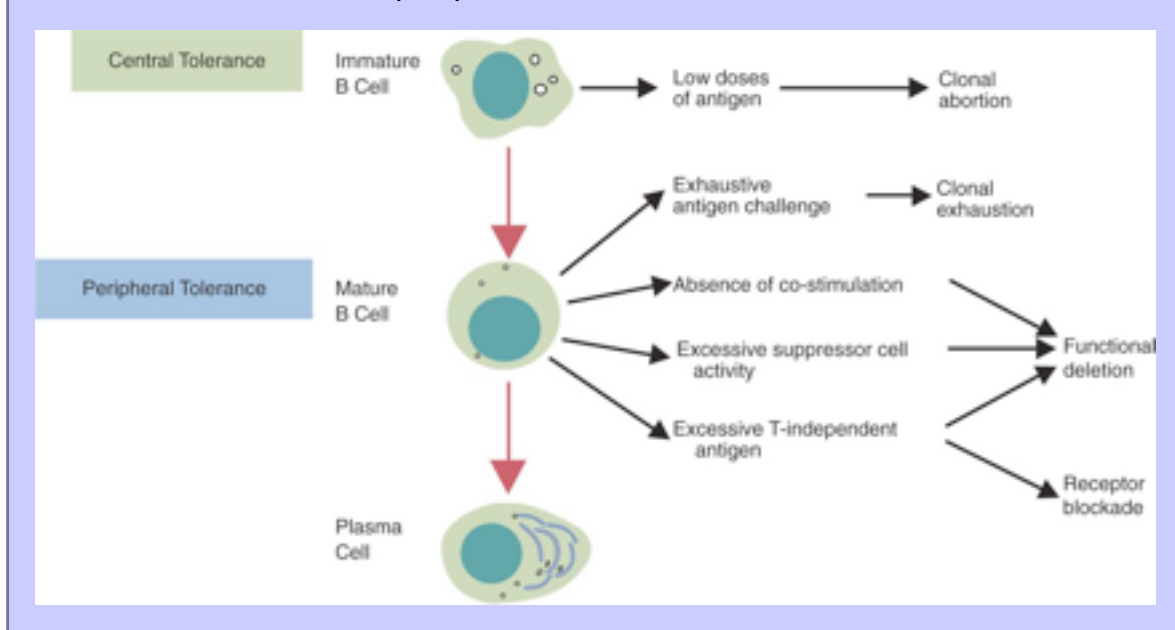


FIGURE 17-8 Central and peripheral tolerance mechanisms in B cells.



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however, and will recover activity fairly rapidly unless steps are taken to maintain tolerance. Self-reactive B cells must also bind to a critical threshold of self-antigen to be made tolerant. This results in selective silencing of the high-affinity B cells. Presumably the failure of low-affinity antiself B cells to become tolerant poses little threat of autoimmune disease because the low-affinity antibodies will not cause tissue destruction.

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B cells subjected to repeated exhaustive antigenic stimulation may differentiate into short-lived plasma cells. If all B cells develop into such plasma cells, no memory B cells will remain to respond to antigen and tolerance will result. Some polymeric antigens such as pneumococcal polysaccharide can bind irreversibly to BCRs, freezing the B cell membrane and blocking any further responses by these cells. The B cells recover once the antigen is removed.

Orally administered proteins may also induce tolerance. The mechanisms depend on the amount of an antigen fed. High doses induce clonal deletion and anergy, whereas lower doses induce the development of regulatory T cells.

17.5 DURATION OF TOLERANCE

The duration of tolerance depends on the persistence of an antigen and on the ability of the bone marrow to generate fresh T or B cells. When an antigen is completely metabolized, tolerance fades. If, however, an antigen is persistent, such as occurs in calf chimeras or with an animal's own self-antigens, then tolerance persists. In the continued presence of an antigen, newly formed antigen-sensitive cells will be killed as soon as their receptors bind self-antigen. Treatment that promotes bone marrow activity, such as low-dose X-irradiation, hastens the fading of tolerance, whereas immunosuppressive drug treatment has the opposite effect.

17.6 CONTROL OF IMMUNE RESPONSES

Tolerance is not the only mechanism of immune regulation employed by the body. The magnitude of immune responses must also be regulated. An inadequate immune response may lead to immunodeficiency and increased susceptibility to infection. An excessive immune response may result in the development of allergies or autoimmunity (see [Chapter 25](#)). Failure to control the lymphocyte proliferation that occurs during immune responses may permit development of lymphoid cell tumors. Failure to control the immune response to the fetus may lead to abortion (see [Chapter 29](#)). The immune responses must therefore be carefully regulated to ensure that they are appropriate in both quality and quantity. As might be anticipated, many different control mechanisms exist.

17.7 ANTIGEN REGULATION OF IMMUNE RESPONSES

Acquired immune responses are antigen driven. They commence only on exposure to an antigen, and once its concentration drops below a critical threshold, they stop. If an antigen persists, the stimulus persists and the immune response is prolonged. Prolonged responses occur after immunization with slowly degraded antigens such as the bacterial polysaccharides, or with antigens incorporated in oil or insoluble adjuvants. T and B cells tend to respond optimally to antigens presented for 3 to 5 days. T cells respond poorly, if at all, to antigens (e.g., self-antigens) that are continuously present in lymphoid organs. Antigens that do not reach organized lymphoid tissues, irrespective of their origin, fail to induce either immunity or tolerance. Thus self-antigens restricted to sites such as the brain, or infectious agents such as papilloma viruses that never enter lymphoid organs, are usually ignored by the immune system.

Antibody responses are also regulated by antigen. Thus rigid polymeric antigens such as those on a bacterial surface or antigens linked to TCR activators such as lipopolysaccharide can induce B cell responses in the absence of T cell help. On the other hand, nonpolymeric, flexible antigens such as soluble proteins induce B cell responses only in the presence of CD4⁺ T cells. Antigen concentration also affects this because the lower the antigen concentration, the greater the need for T cell help.

17.7.1 Antigen Processing and Immune Regulation

The nature of the immune response may vary in different parts of the body as a result of processing by different dendritic cell populations. Langerhans cells seem especially suited for promoting T cell responses, whereas follicular dendritic cells prime B cells. DC1 cells are optimized to present antigens to Th1 cells, whereas DC2 cells present antigens to Th2 cells. Adjuvants also influence the type of immune response through their effects on antigen-presenting cells (see [Chapter 20](#)). Thus lipids conjugated to protein antigens commonly induce cell-mediated responses rather than antibody production and localize in T cell, rather than B cell, areas of lymphoid tissues.

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17.8 ANTIBODY REGULATION OF IMMUNE RESPONSES

Antibodies generally suppress immune responses. IgG antibodies tend to suppress the production of both IgM and IgG, whereas IgM antibodies tend to suppress only the synthesis of IgM. Specific antibodies tend to suppress a specific immune response better than nonspecific immunoglobulins. An excellent example of this is seen in the method employed to prevent hemolytic disease of the newborn in humans (see [Chapter 26](#)). In this disease a mother who lacks the Rhesus (Rh) antigen makes antibodies against Rh antigens on the red blood cells of her fetus. If the

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mother is given antibodies against this antigen at the time of her exposure to fetal red blood cells at birth, she will be completely prevented from responding to this antigen.

This negative feedback of antibodies on B cells is mediated through the BCR and CD32b (FcγRIIb). In diseases where serum immunoglobulin levels are abnormally high, as in patients with myelomas (see [Chapter 13](#)), this feedback depresses normal antibody synthesis and patients become susceptible to infections. A similar phenomenon occurs in newborn animals that acquire antibodies from their mother. The presence of these maternal antibodies, while conferring protection, inhibits immunoglobulin synthesis and so prevents the successful vaccination of newborn animals ([Figure 17-9](#)).

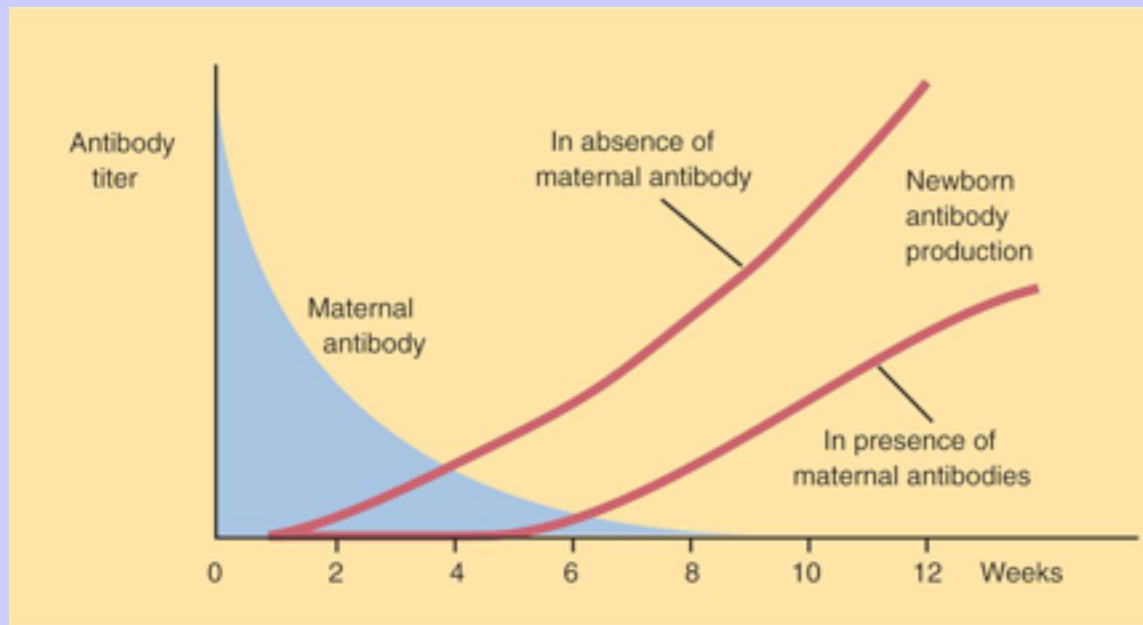
Serum IgG levels are also regulated through the FcRn immunoglobulin receptor. FcRn is widely distributed on endothelial cells in muscle, vasculature, and hepatic sinusoids. Immunoglobulins that bind to FcRn are protected from degradation. If FcRn expression remains constant, IgG levels remain stable. If IgG levels rise, the surplus will fail to bind FcRn and be degraded. Conversely, if IgG levels drop, a greater proportion will bind to FcRn and be protected.

The class, as well as the quantity, of immunoglobulins produced during an immune response is also regulated. Most unstimulated B cells express both IgM and IgD BCRs. During an immune response, these cells switch to the production of IgM, IgG, IgA, or IgE. This class switch is controlled by helper T cells. In animals given T-independent antigens, there is no class switch and a persistent low-level IgM response ensues (see [Chapter 11](#), [Figure 11-12](#)). Neonatal bursectomy in birds may also block the IgM-to-IgG switch.

17.9 INHIBITORY RECEPTORS

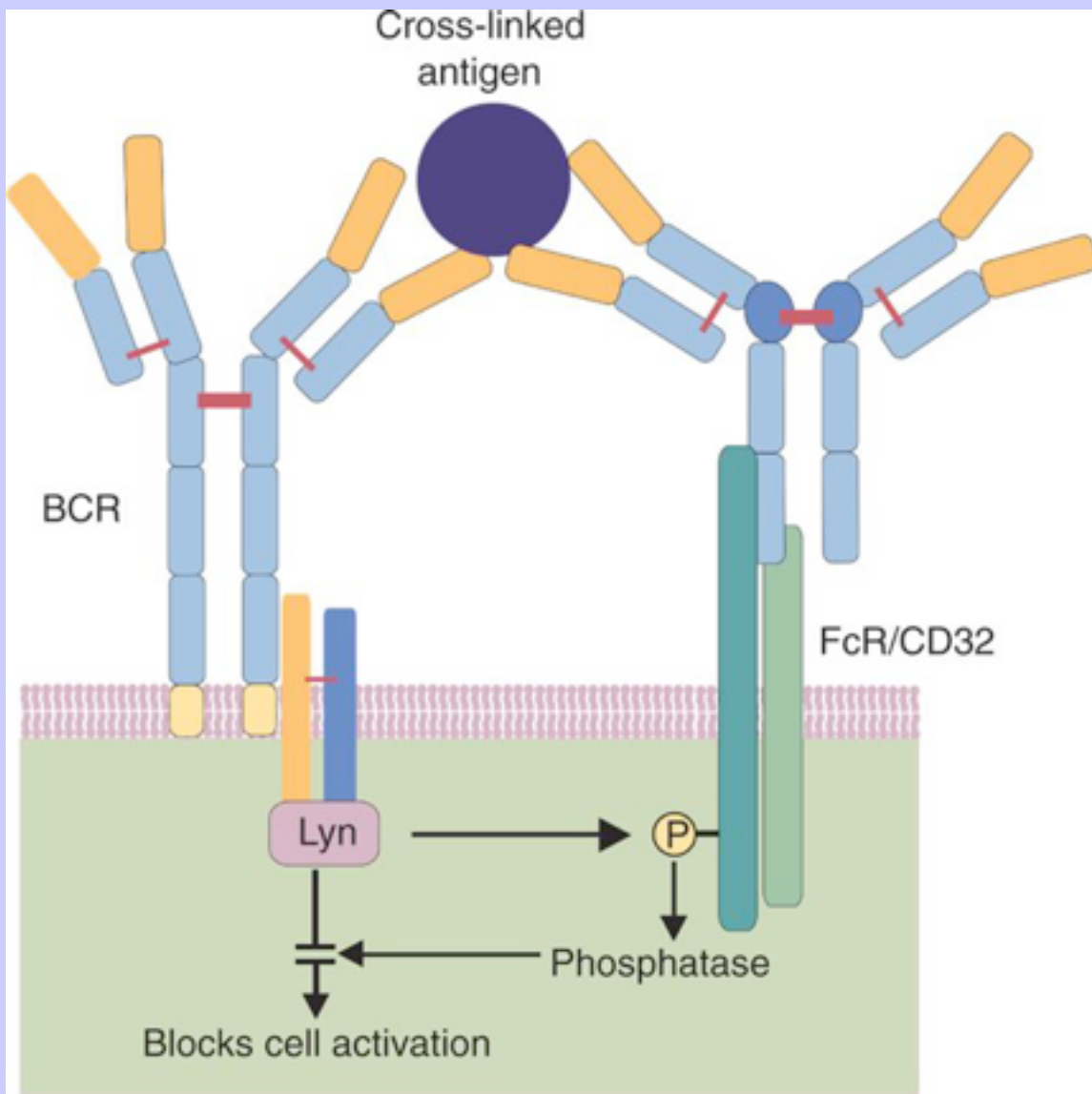
A key feature of the acquired immune system is that, while it is poised to launch a potent array of destructive mechanisms against invaders, the body maintains control of the process. It is critically important to limit and eventually terminate a response by inactivating or eliminating pathways that are no longer required. This regulation involves the extensive use of inhibitory receptors. These are especially important in diminishing the activity of lymphocytes once they have completed their task. They provide a crucial safeguard against inappropriate immune responses. Thus activation and inhibition must be paired to initiate and terminate immune responses. In some cases activating and inhibitory receptors recognize similar ligands, so that the net outcome is a product of the relative strength of these signals. Loss of inhibitory

FIGURE 17-9 The presence of maternal antibody in a newborn animal effectively delays the onset of immunoglobulin synthesis through a negative feedback process.



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FIGURE 17-10 Cross-linkage of a B cell receptor (BCR) with antibody with CD32, an Fc receptor, by antibody and antigen can turn off a B cell by activating a phosphatase that in turn blocks signaling by tyrosine kinase.



signals is often associated with autoimmunity or hypersensitivity.

17.9.1 B Cell Inhibitory Receptors

An excellent example of an inhibitory receptor is CD32b (FcγRIIb). This receptor is expressed on B cells. Any antibodies present will occupy these receptors. If these receptor-bound antibodies are linked to a BCR through an

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antigen, the BCR and CD32 will be drawn close together ([Figure 17-10](#)). As a result, their signal transduction pathways interact and BCR signal transduction is blocked. This prevents B cell activation and triggers its apoptosis. This pathway serves as a feedback mechanism whereby B cell activation is suppressed by antibody and so prevents uncontrolled B cell responses. Since another receptor, FcγRIII, stimulates B cells, B cell responses can be controlled by regulating the ratio of FcγRIIb to FcγRIII. Macrophage activation is regulated in a similar manner, and activated macrophages have a high FcγRIII-to-II ratio. Other B cell inhibitory receptors include CD5, CD22, CD66a, and CD72. A deficiency of any of these results in uncontrolled B cell proliferation.

17.9.2 T Cell Inhibitory Receptors

CD28 and CTLA4 on T cells both bind the same ligand (CD80) but deliver antagonistic signals. CD28 is an activator, whereas CTLA4 is an inhibitor. A deficiency of CTLA4 leads to uncontrolled T cell proliferation and autoimmunity.

17.10 REGULATORY CELLS

Although much immune regulation is “passive” in that self-reactive lymphocytes are eliminated by central tolerance, it is clear that regulatory cells in peripheral tissues also “actively” regulate the immune system. Cells known to have a regulatory function include T cells, macrophages, dendritic cells, NKT cells, and natural suppressor cells.

17.10.1 Regulatory T Cells

The immune system has evolved a network of regulatory T cells (T_{reg} cells). T_{reg} cells play a master role in regulating the immune system and maintaining the balance between peripheral tolerance and immunity. They make up roughly 5% of circulating T cells and 10% of lymph node T cells in the dog. Some of these T_{reg} cells are naturally occurring, while others must be induced by cytokine exposure. Natural T_{reg} cells differentiate in the normal thymus as a functionally distinct cell subset with a broad TCR repertoire. T_{reg} cells express CD4 and CD25 (the α chain of the IL-2 receptor). (All activated T cells express CD25, but T_{reg} cells are the only ones that express it when naïve.) They also express the transcription factor FoxP3, which controls their development. Natural T_{reg} cells may either act by secreting suppressive cytokines or they may act through direct cell-cell contact. Thus T_{reg} cells can secrete the two major suppressive cytokines, IL-10 and transforming growth factor- β (TGF- β). These T_{reg} cells can suppress the proliferation of helper T cells in response to an antigen and prevent experimental autoimmune disease. They prevent inappropriate T cell activation in the absence of an antigen. When an animal is infected, activated dendritic cells secrete IL-6, which overrides the suppressive effects of T_{reg} cells and permits an effector T cell response to occur. T_{reg} cells can also suppress CD4 and CD8 T cell responses by pathways independent of IL-10 and TGF- β . The mechanism is unclear, but it may act by reverse signaling through CD80. T_{reg} cells may also be cytotoxic for responder T cells and kill them using both perforins and granzymes.

Oral administration of an antigen may induce the appearance of T_{reg} cells. Thus T_{reg} cells from the mesenteric lymph nodes of orally tolerant animals secrete TGF- β , with various amounts of IL-4 and IL-10. It has long been recognized that the activities of regulatory T cells have been controlled by cytokines. Because T_{reg} cells characteristically express CD25, IL-2 regulates their development. Lymphoproliferation followed by lethal autoimmunity develops in IL-2-deficient mice. T_{reg} cells are also influenced by signals mediated through TLRs.

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They express TLR1, 2, 5, 7, and 8 and possibly TLR4. TLR2-deficient mice have very few T_{reg} cells. In general, it appears that TLR triggering tends to inhibit the suppressive effects of these cells and so promote immune responses.

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T_{reg} cells are not the only agents of cellular control of immune responses. Many of the regulatory activities of T cells reflect the antagonistic functions of Th1 and Th2 cells. For example, IFN- γ from Th1 cells can suppress IgE production whereas IL-10 from Th2 cells is suppressive for dendritic cell IL-12 production and thus for the production of cytokines by Th1 cells. Likewise IL-4 may suppress IL-2-mediated B cell proliferation.

CD8⁺ T cells can also secrete cytokine mixtures typical of Th1 or Th2 cells, so that a CD8⁺ cell secreting IL-10 could be an effective suppressor cell. Another possible suppressive mechanism involves the stimulation of cytotoxic T cells by antigen presented on B cells. Since some cytotoxic T cells are MHC class II restricted, they might kill B cells presenting antigens in the conventional manner.

17.10.1.1

Interleukin-10

IL-10 was first described as an inhibitor of cytokine synthesis produced by Th2 cells. However, it is now clear that dendritic cells, macrophages, and natural killer (NK) cells can also produce IL-10. It is produced in large amounts by T_{reg} cells and by M2 macrophages. IL-10 can inhibit the production of many cytokines, including IL-1 α , IL-1 β , IL-5, IL-6, CXCL8, IL-12, tumor necrosis factor- α (TNF- α), granulocyte-macrophage colony-stimulating factor, and granulocyte colony stimulating factor (G-CSF). It downregulates MHC class II and co-stimulatory molecule expression on dendritic cells and macrophages and hence impairs their antigen-presenting abilities. IL-10 selectively inhibits the CD28 co-stimulatory pathway by blocking CD28 phosphorylation. As a result it inhibits the synthesis of the Th1 cytokines IL-1, IFN- γ , and TNF- α . IL-10 also suppresses the secretion of IL-1, IL-6, TNF- α , and oxidants by macrophages. It downregulates MHC class II expression and stimulates production of IL-1RA, an antiinflammatory cytokine. IL-10 downregulates the production of IFN- γ and TNF- α by NK cells. IL-10 inhibits not only Th1 but also many Th2 responses. IL-10 or IL-10-treated dendritic cells can induce a long-lasting, antigen-specific, anergic state when both CD4⁺ and CD8⁺ T cells are activated in its presence. It is interesting to note that pigs that become tolerant to a foreign kidney graft possess T cells producing unusually large amounts of IL-10.

17.10.1.2

Transforming Growth Factor- β

TGF- β s are a family of five glycoproteins, three of which (TGF- β 1, TGF- β 2, and TGF- β 3) are found in mammals. Two others (TGF- β 4 and TGF- β 5) have been described in chickens and *Xenopus* toads. They are secreted as an inactive or latent molecule and subsequently activated. They are produced by platelets, activated macrophages, neutrophils, B cells, and T cells and act on most cell types, including T and B cells, dendritic cells, macrophages, neutrophils, and fibroblasts. The TGF- β s regulate cell division and are immunosuppressive.

TGF- β regulates the growth, differentiation, and function of all classes of lymphocytes, dendritic cells, and macrophages. In general, TGF- β inhibits T and B cell proliferation and stimulates their apoptosis, effectively acting as an immunosuppressive molecule. Apoptotic T cells release TGF- β , thus contributing to the suppressive environment. A subset of CD4⁺ T cells can act as regulatory cells by secreting large amounts of TGF- β . These have been called Th3 cells and may play a role in some forms of tolerance. TGF- β influences

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the differentiation of Th subsets. It tends to promote Th1 responses and the production of IL-2 in naïve T cells, but it also antagonizes the effects of IFN- γ and IL-12 on memory cells.

TGF- β is required for optimal dendritic cell development and regulates the interaction between follicular dendritic cells and B cells. It also controls the development and differentiation of B cells, inhibiting their proliferation, inducing apoptosis, and regulating the switching of B cells to IgA production.

TGF- β is produced by macrophages and regulates their activities. It can be either inhibitory or stimulatory, depending on the presence of other cytokines. Thus it can enhance integrin expression, as well as phagocytosis by blood monocytes. On the other hand, it suppresses the respiratory burst and nitric oxide production. It blocks monocyte differentiation and the cytotoxic effects of activated macrophages.

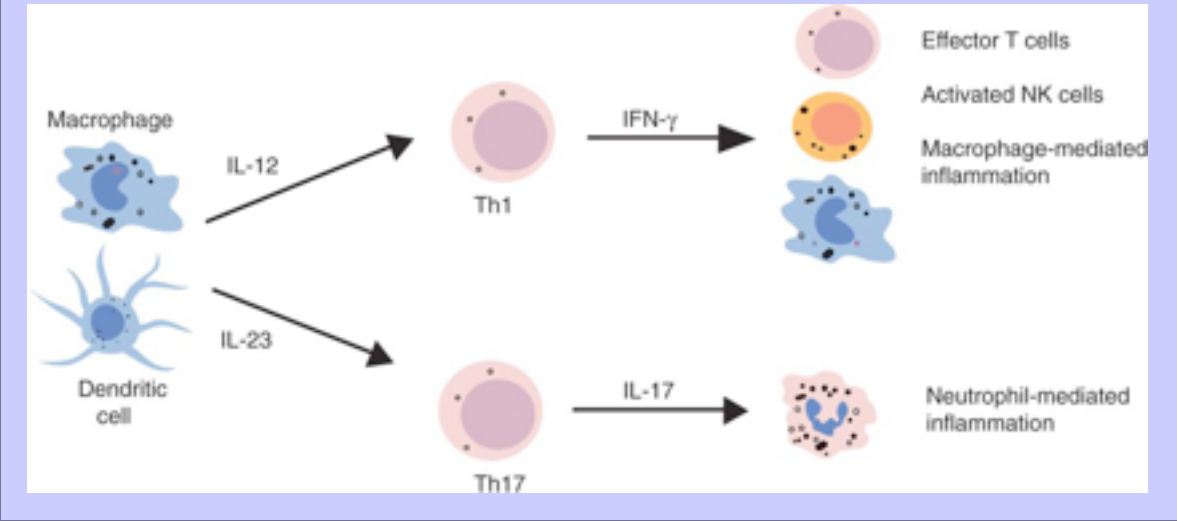
17.10.2

Th17 Cells

While Th1 and Th2 cells are central to the regulation of acquired immunity, it has been found that a third population of helper cells, called Th17 cells, regulates innate immunity, specifically inflammation ([Figure 17-11](#)). For example, in chronic inflammation, dendritic cells and macrophages activated through TLR2 secrete IL-23 rather than IL-12. (IL-23 is related to IL-12 since they share a common chain called p40.) IL-23 promotes the survival and activation of Th17 cells. These Th17 cells develop in the presence of TGF- β and IL-6 and are opposed by Th1 cytokines. Th17 cells in turn secrete IL-17, a cytokine mixture that regulates inflammation and autoimmunity, as well as antibacterial and antifungal immunity. This IL-23/IL-17 axis plays a key role in recruiting neutrophils to sites of acute infection and tissue damage. The IL-17 family contains several

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FIGURE 17-11 The generation of Th1 and Th17 cells depends on the production of interleukin-12 (IL-12) or IL-23 by antigen-presenting cells. Th1 cells promote cell-mediated responses, and Th17 cells promote innate responses.



related proteins with similar biological properties. IL-17 promotes inflammatory responses by triggering the production of the proinflammatory cytokines IL-1, IL-6, and TNF- α , as well as CXCL8 and CXCL1 by endothelial cells, epithelial cells, and macrophages. IL-17 also promotes the production of G-CSF by stromal

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cells and so promotes the accumulation of neutrophils at invasion sites. Thus IL-17 links T cell activation and the mobilization of neutrophils. Th17 cells also secrete IL-22, which acts on cells of the skin and digestive and respiratory systems to increase expression of several β -defensins and presumably promotes innate immunity in these tissues.

IL-27 is a member of the IL-12 family related to both IL-12 and IL-23. It too is produced by dendritic cells and macrophages. Originally reported to promote Th1 responses, IL-27 is now known to be a regulatory cytokine that suppresses Th1, Th2, and Th17 and also inhibits neutrophil migration and the respiratory burst. IFN- γ from Th1 cells suppresses the development of Th17 cells and so inhibits IL-17-mediated inflammation.

17.10.3 Regulatory Macrophages

Activation of macrophages by IFN- γ leads to the development of proinflammatory classically activated M1 cells. However, exposure of macrophages to IL-4 or IL-13 leads to the development of M2 cells. These M2 cells participate in tolerance induction, suppress inflammation, and participate in tissue repair. M2 cells increase expression of the macrophage mannose receptor, the β -glucan receptor, and CD163; enhance endocytosis and antigen processing; and increase MHC II expression. M2 macrophages produce large amounts of antiinflammatory cytokines such as IL-10, TGF- β , and IL-1RA. They do not show enhanced killing because IL-4 and IL-13 promote the production of arginase, which generates ornithine rather than nitric oxide (see [Chapter 16, Figure 16-16](#)).

In healthy animals, M2 cells may be found in the placenta and lung, where they inhibit unwanted inflammatory reactions. Thus placental and alveolar macrophages inhibit dendritic cell antigen presentation and can inhibit mitogen responses in lymphocytes. These regulatory macrophages are responsible for control of granuloma formation as well as for skin tolerance induced by UVB radiation. They can also be found in healing tissues, where they are associated with angiogenesis.

17.10.3.1 IDO and Tolerance

Various cell types including dendritic cells, some macrophages, fibroblasts, trophoblast giant cells, endothelial cells, and some tumor cell lines may produce indoleamine 2,3-dioxygenase (IDO). This enzyme catalyzes the oxidative degradation of the essential amino acid tryptophan and as a result causes local depletion of this amino acid and tryptophan-free zones. Tryptophan is an essential amino acid found in many proteins. T cells undergo cell cycle arrest and apoptosis when deprived of tryptophan. Th1 cells appear to be more sensitive to tryptophan depletion than Th2 cells. IDO therefore serves a potent inhibitor of T cell activation, proliferation, and survival within tissues.

Certain T_{reg} cells may induce IDO expression in other cells. Thus T_{reg} cells may be responsible for regulating IDO expression in the trophoblast. Likewise, subpopulation of human dendritic cells has been shown to produce IDO. These seem to be mainly plasmacytoid dendritic cells and cells that express CD8a. Physiological IDO activity has been documented in T cell tolerance to tumors, as a negative regulator in autoimmune diseases, and in some experimental allergies. Most importantly, IDO plays a key role in preventing immunological rejection of the fetus and of liver and corneal allografts (see [Chapter 29](#)). IDO can also act as a defensive enzyme, since by destroying tryptophan it prevents the growth of *Toxoplasma gondii*, *Chlamydia pneumoniae*, streptococci, and mycobacteria.

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17.10.4 Tolerogenic Dendritic Cells

In the thymus, dendritic cells may promote tolerance by destroying self-reactive T cells. In lymphoid organs, the normal function of dendritic cells is to capture and process foreign antigens for presentation to T cells. However, the precise signals generated by dendritic cells depend on their state of maturity, on their display of co-stimulating molecules, and on the presence or absence of inflammatory molecules. Thus proteins from dead and dying cells that are captured by immature dendritic cells in the absence of inflammation may cause dendritic cells to trigger apoptosis in responding T cells or cause the T cells to differentiate into IL-10–producing regulatory cells. Treatment of dendritic cells with IL-10 can block their ability to activate Th1 cells while preserving their ability to promote Th2 responses.

17.10.5 Natural Suppressor Cells

Natural suppressor (NS) cells are large granular lymphocytes that secrete proteins that have suppressor cell–inducing activity. They suppress B and T cell proliferation as well as immunoglobulin production. NS cells occur normally in the adult bone marrow and neonatal spleen and possibly regulate innate immune responses. Potent NS activity develops in animals under-going a graft-versus-host disease (see [Chapter 29](#)).

17.10.6 When Do Regulatory Cells Work?

Regulatory cell activities have been described as regulating almost all aspects of immune reactivity. Regulatory T cells, for example, work constantly throughout an animal's life to prevent self-reactivity. They are responsible for lack of immune responses in the newborn; immunosuppression following trauma, burns, or surgery; prevention of autoimmunity; some cases of hypogammaglobulinemia; and blocking of responses to mitogens. Regulatory cells are found in some tumor-bearing animals, where they block tumor rejection, and in pregnant animals, where they block rejection of the fetus.

17.11 REGULATION OF APOPTOSIS

The thymus of the mouse releases about a million new T cells into the circulation every day. Presumably a cow would produce many more. In order to keep the number of lymphocytes in the mouse body relatively constant, a million must also die. Likewise, the mouse bone marrow releases about 10^7 B cells daily and an equivalent number must die. In addition, lymphocytes divide in response to antigens. To keep the number of lymphocytes constant, all this proliferation must be balanced by the removal of cells by apoptosis. Apoptosis also removes autoreactive lymphocytes and limits the clonal expansion of lymphocytes during an immune response. This homeostatic system is carefully tuned because, if it fails, excess lymphocytes can cause lymphoid tumors or autoimmunity. The regulation depends on providing cell populations with survival signals. If these are inadequate, cells will die. These regulatory signals probably differ among subpopulations since the loss of some cells, such as naïve T cells, is not compensated for by an increase in memory T cells. These signals are likely provided by cytokines such as IL-2, IL-4, IL-9, and IL-21.

Apoptosis in lymphocytes is mediated by intracellular proteases. The best characterized of these are cysteine proteases belonging to the caspase family. These proteases are constitutively expressed as inactive precursors in lymphocytes. Proteins of the bcl-2 family modulate their activity. Thus, in a quiescent cell, survival depends on the ongoing presence of bcl-2. Animals lacking bcl-2 progressively lose lymphocytes and become immunodeficient. Cell receptors also regulate apoptosis. Thus survival is signaled through IL-2, IL-4, IL-7, and IL-15 receptors

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whereas cell death is signaled through Fas (CD95) and TGF- β . Adherence receptors such as integrins also regulate the survival of quiescent B and T cells. Thus if a lymphocyte does not traffic to the lymphoid organs, it may be destroyed by apoptosis. Lymphoid organs contain adhesion receptor ligands that allow long-term lymphocyte survival. Thus together cytokine and adhesion receptors ensure the survival of quiescent lymphocytes.

Once a lymphocyte has been activated, it becomes less likely to undergo apoptosis unless it gets inappropriate or conflicting signals. The stronger the antigenic signals, the greater the resistance to apoptosis. However, activated lymphocytes also become more susceptible to killing through the TNF receptors and CD95. Activation of T cells by antigen causes expression of CD95L. As a result, they become sensitive to CD95-mediated killing. However, the CD95 pathway is normally blocked by stimulatory signals such as those transmitted through CD28 on T cells and CD40 on B cells. If an antigen or co-stimulatory signal is lost, the activated cell becomes sensitive to CD95-induced apoptosis. This is why lymphocytes are eliminated at the end of the immune response. Lymphocyte activation, while temporarily protecting lymphocytes against apoptosis, will also ensure that these cells can eventually be removed. Thus CD40 upregulates CD95 on B cells and IL-2 primes T cells for subsequent killing by CD95L.

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17.12 NEURAL REGULATION OF IMMUNITY

The central nervous and immune systems communicate extensively with each other. The central nervous system communicates with the immune system through parasympathetic and sympathetic nerves and by soluble neurotransmitters. Neuroendocrine hormones such as corticotrophin-releasing factor and α -melanocyte-stimulating hormone regulate cytokine balances. Conversely, the immune system modulates central nervous system activities such as appetite, temperature, and sleep behaviors.

17.12.1 Stress

It has been known for many years that mental attitudes, especially stress, influence resistance to infectious diseases ([Figure 17-12](#)). One obvious example is shipping fever. This is a complex pneumonia of cattle primarily caused by several viral respiratory pathogens with secondary infection by *Mannheimia hemolytica*. It develops in cattle that have been transported in confined spaces for long distances (and hence, many hours) with minimal feed and water and usually after rapid weaning and castration. The stress involved in the shipping process is sufficient to make these cattle highly susceptible to pneumonia. Stress can depress T cell responses, NK activity, IL-2 production, and expression of IL-2R on lymphocytes. Reduction in stress can have a reverse effect.

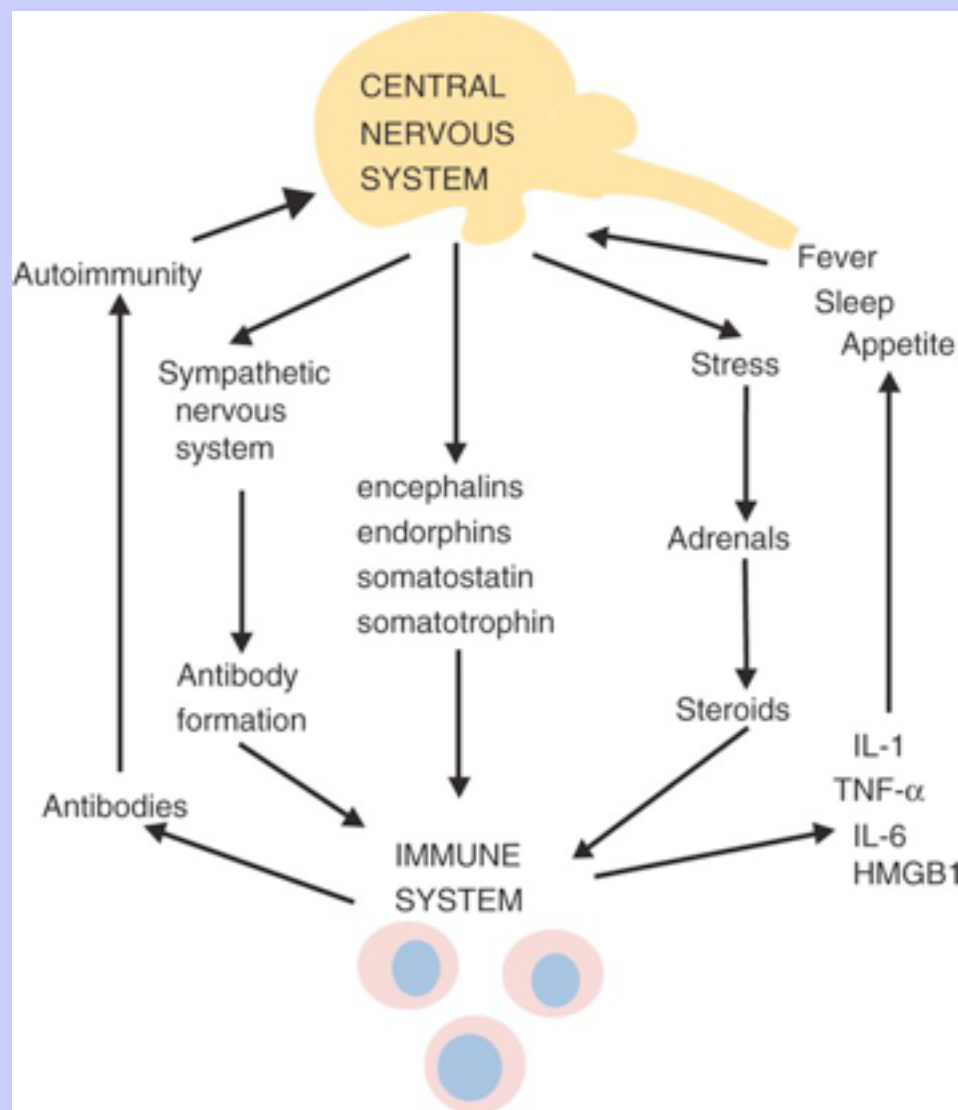
Stress can be due to something as simple as early weaning, which reduces IL-2 production in piglets. Stress in pregnant sows results in immunosuppression of their offspring. Thus confinement stress late in pregnancy results in the birth of piglets whose T and B cells have a reduced ability to respond to mitogens. Both morbidity and mortality are increased in piglets from stressed sows. A different form of stress may result from mammalian social structures. A dominance hierarchy regulates many mammalian populations. The rank of an individual animal within such a hierarchy influences its quality of life. Depending on the way the hierarchy is established, some members may be very highly stressed. Animals of high rank will be stressed if maintaining dominance requires constant fighting. This occurs, for example, in wild dogs, lemurs, and mongooses. In hierarchies where dominant members intimidate through psychological intimidation, such as in mice, rats, and many monkeys, low-ranking individuals may be stressed and immunosuppressed. If new individuals are introduced into a group, or a dominant animal loses its position, stresses occur as a result of the reorganization. In pigs, it has been shown that there is a relationship between social status and disease susceptibility. Thus morbidity and mortality among pigs challenged with pseudorabies virus are highest among subordinate animals. Dominant pigs have lymphocytes that are more responsive to virus antigens. This of course makes sense from an evolutionary point

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of view in that the least reproductively fit animals were more likely to die of disease, but it is difficult to separate cause and effect in this phenomenon. Were subordinate animals immunosuppressed because they were under stress as a result of their lowly status? Alternatively, could it be that those animals with a highly effective immune system were healthier and thus better able to reach high social status within the population? Were dominant animals better fed than subordinates? Certainly high levels of social stress are found in confined, crowded animal populations.

Behaviorally pigs can be divided into two groups: aggressive animals that tend to fight other animals and then may flee rapidly, and passive animals that tend to cope with stress by withdrawing gradually from stressful situations. These differences are associated with different behavioral, physiological, and endocrine responses to stress. Thus aggressive pigs have higher in vitro and in vivo cell-mediated immune responses

FIGURE 17-12 Some of the ways in which the central nervous system and the immune system interact.



but lower humoral responses than passive animals. This suggests that there are differences in their relative Th1 and Th2 responses. However, when these animals are stressed, the aggressive ones show a much greater drop in these responses than the passive animals. Thus differences in the way animals cope with stress are reflected in differences in immune reactivity. This stress effect is clearly mediated by the products of the hypothalamic-pituitary-adrenal cortical axis and by the sympathetic-adrenal medullary axis. For example, the adrenal cortex is stimulated by adrenocorticotrophic hormone from the pituitary under the influence of corticotrophin-releasing hormone from the hypothalamus. As a result, glucocorticoids are secreted and suppress T cell function by blocking the NF- κ B pathway. Other mechanisms may involve noradrenaline signaling leading to NF- κ B activation in mononuclear cells. It may also involve direct signaling from nerves in lymphoid organs. Cells of the immune system have receptors for the opioid peptides. Neuropeptides such as enkephalins and endorphins are released during stress. These can bind to receptors on lymphocytes and influence their activity. Thus the generation of cytotoxic T cells is enhanced by met-enkephalin and β -endorphin while α -endorphin suppresses antibody formation and β -endorphin reverses this suppressive effect. Other neuropeptides that influence the immune system include adrenocorticotrophic hormone, oxytocin, vasoactive intestinal peptide, somatostatin, prolactin, and substance P. Certain sites in the brain influence immune function by controlling neurotransmitter function or the autonomic nervous system.

17.12.2 Innervation

Many lymphoid organs are well supplied by nerves through the autonomic nervous system. The parasympathetic nerves link to lymphoid organs through the neurotransmitter acetylcholine. Thus vagal nerve stimulation suppresses the systemic shock response to endotoxin by downregulating hepatic TNF- α synthesis. Stimulation of neurons in dorsal root ganglia results in vasodilatation. Activation of acetylcholine receptors on macrophages inhibits production of IL-1 and TNF- α . The sympathetic nerves act through the neurotransmitter norepinephrine. They innervate the thymus, the splenic white pulp, and the lymph nodes. They influence blood flow, vascular permeability, and lymphocyte migration and differentiation. For example, nerves are linked to Langerhans cells in the skin. By releasing neuropeptides, these nerves can depress the antigen-presenting ability of these Langerhans cells. This might explain why “hot spots” in dogs worsen with anxiety. Surgical or chemical sympathectomy of the spleen enhances antibody production and can induce changes in the distribution of lymphocyte subpopulations. Thus NK cell activity appears to be modulated directly by the preoptic nucleus of the hypothalamus through the splenic nerve. Following tissue damage denervated skin shows reduced inflammation and heals more slowly.

The sympathetic nervous system can alter the Th1/Th2 balance through the β -adrenergic receptor. Propranolol, a β -adrenergic antagonist, prevents the macrophage-mediated release of IL-10. Stimulation of sympathetic nerves enhances production of Th2 cytokines while inhibiting production of Th1 cytokines. Norepinephrine suppresses production of IL-6 and TNF- α . Many neuropeptides, such as vasoactive intestinal peptide and neurokinin-1, have a similar structure to the antimicrobial peptides so that many also have antimicrobial properties and may be involved in host defense. For example, neurokinin-1 (NK-1, also known as substance P) is a neurotransmitter peptide involved in pain and inflammation but it also has significant antibacterial activity. Other neuropeptides have similar effects. As a result, appropriate nervous stimulation can promote neuropeptide release that enhances local antibacterial activity. Thus the pain associated with acute inflammation may well reflect local resistance to infection.

Immune responses are also modulated by environmental factors. Thus changes in day length (photo-period) influence immune responses. These effects can be complex, but in general reduced day length appears to promote immune reactivity. The effect appears to be mediated through the hormone melatonin.

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Finally, the innate immune system can influence nervous function. For example, cytokines such as IL-1, IL-6, and TNF- α induce “sickness behavior,” including fever, fatigue, depressed activity, and excessive sleep. All these are closely associated with the immune response to infectious agents and chronic inflammation.

17.13

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18 CHAPTER 18 Immunity in the Fetus and Newborn

18.1 KEY POINTS

- The immune system is fully formed at birth but has never been used. Hence all acquired immune responses in the newborn are slow primary responses.
- Newborn mammals obtain immunoglobulins from their mother either by direct transfer across the placenta, as in primates, or by ingestion of immunoglobulin-rich colostrum immediately after birth.
- Failure of this passive transfer may result in the newborn animal suffering from overwhelming infections.
- Milk provides a constant supply of immunoglobulin (IgA in most species), which helps protect the newborn against intestinal infections.
- Vaccination protocols in newborn animals must take into account their inability to induce antibody responses in the presence of persistent maternal immunity.

When a mammal is born, it emerges from the sterile uterus into an environment where it is immediately exposed to a host of micro-organisms. Its surfaces, such as the gastrointestinal tract, eventually develop a dense, complex microbial flora. If it is to survive, the newborn animal must therefore be able to control this microbial invasion. In practice, the acquired immune system takes some time to become fully functional and innate mechanisms are responsible for the initial resistance to infection. In some species with a short gestation period, such as mice, the acquired immune system may not even be fully developed at birth. In animals with a long gestation period, such as the domestic mammals, the acquired immune system is fully developed at birth but cannot function at full adult levels for several weeks. The complete development of acquired immunity depends on antigenic stimulation. The proper development of B cells and B cell antigen receptor (BCR) diversity requires clonal selection and antigen-driven cell multiplication (see [Chapter 13](#)). Thus newborn mammals are vulnerable to infection for the first few weeks of life. They need assistance in defending themselves at this time. This temporary help is provided by the mother in the form of antibodies and possibly T cells. The passive transfer of immunity from mother to newborn is essential for survival.

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18.2 DEVELOPMENT OF THE IMMUNE SYSTEM

The development of the immune system in the mammalian fetus follows a consistent pattern. The thymus is the first lymphoid organ to develop, followed closely by the secondary lymphoid organs. B cells appear soon after the development of the spleen and lymph nodes, but antibodies are not usually found until late in fetal life, if at all ([Box 18-1](#)). The ability of the fetus to respond to antigens develops very rapidly after the lymphoid organs appear, but all antigens are not equally capable of stimulating fetal lymphoid tissue. The immune system develops in a series of steps, each step permitting the fetus to respond to more antigens. These steps are driven by a gradual increase in the use of gene conversion or somatic mutation to increase antibody diversity. The ability to mount cell-mediated immune responses develops at the same time as antibody production. Data from humans suggest that T cell receptor diversity is limited in the fetus and neonate and that cytokine production may be low. This may simply be due to their lack of exposure to foreign antigens.

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18.2.1

Specific Animal Immune Systems

18.2.1.1

Foal

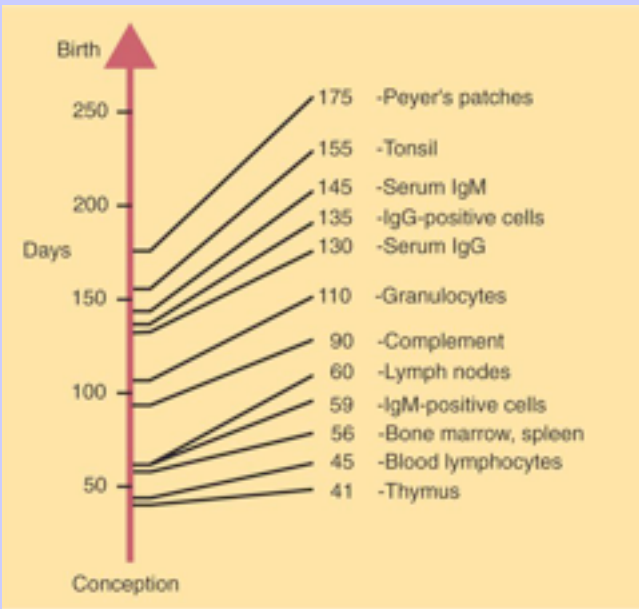
The gestation period of the mare is about 340 days. Lymphocytes are seen first in the thymus around 60 to 80 days postconception. They are found in the mesenteric lymph node and intestinal lamina propria at 90 days and in the spleen at 175 days. Blood lymphocytes appear at around 120 days. A few plasma cells may be seen at 240 days. Graft-versus-host disease, a cell-mediated response, has developed in immunodeficient foals transplanted with tissues from a 79-day-old fetus. The equine fetus can respond to coliphage T2 at 200 days postconception and to Venezuelan equine encephalitis virus at 230 days. Newborn foals have detectable quantities of immunoglobulin M (IgM) and IgG and occasionally IgG3 in their serum, but IgE production in the horse does not begin until foals are 9 to 11 months of age. Like other large herbivores, the foal has a well-developed ileal Peyer's patch that serves as a primary lymphoid organ and eventually involutes.

18.2.1.2

Calf

The immune system of the calf develops early in fetal life. Although the gestation period of the cow is 280 days, the fetal thymus is recognizable by 40 days postconception. The bone marrow and spleen appear at 55 days. Lymph nodes are found at 60 days, but Peyer's patches do not appear until 175 days (Figure 18-1). Peripheral blood lymphocytes are seen in fetal calves by day 45, IgM⁺ B cells by day 59, and IgG⁺ B cells by day 135. The time of appearance of serum antibodies depends on the sensitivity of the techniques used. It is therefore no accident that the earliest detectable immune responses (identified using highly sensitive virus neutralization tests) are those directed against viruses. Fetal calves have been reported to respond

FIGURE 18-1 Progressive development of the immune system in the fetal calf.



to rotavirus at 73 days, to parvovirus at 93 days, and to parainfluenza 3 virus at 120 days. Fetal blood lymphocytes can respond to mitogens between 75 and 80 days, but this ability is temporarily lost around the time of birth as a result of high serum steroid levels. T cell subpopulations are present in calves at levels comparable to adults, but B cell numbers increase significantly during the first six months after birth.

18.2.1.2.1

Box 18-1 Immunity in Marsupials

Although the immune responses of marsupials are usually slower to develop than those of placental mammals, their immune system may develop remarkably early. Thus the opossum, *Monodelphis domestica*, is born after only 15 days gestation and newborn opossums have neither immunological tissues nor organs. Nevertheless, young opossums can make antibodies by 7 days postpartum. During their first 7 days of life they rely totally on passive immunity from their mother's milk and suckle permanently until 16 days. They suckle intermittently after that and are weaned at 60 days, when absorption of antibodies across the intestinal epithelium ceases.

18.2.1.3

Lamb

The gestation period of the ewe is about 145 days. Major histocompatibility complex (MHC) class I positive cells can be detected by day 19, and MHC class II positive cells can be found by day 25. The thymus and lymph nodes are recognizable by 35 and 50 days postconception, respectively. Gut-associated follicles appear in the colon at 60 days, jejunal Peyer's patches around days 75 to 80, and ileal Peyer's patches at days 110 to 115. Blood lymphocytes are seen in fetal lambs by day 32, and CD4⁺ and CD8⁺ cells appear in the thymus by 35 to 38 days. B cells are detectable at 48 days in the spleen and by that time have already begun to rearrange their Vg genes. C3 receptors appear by day 120, but Fc receptors do not appear until the animal is born. Fetal liver lymphocytes can respond to phytohemagglutinin by 38 days. Lambs can produce antibodies to phage fx 174 at day 41 and reject skin allografts by day 77. Some fetal lambs can produce antibodies to Akabane virus by as early as 50 days postconception. Antibodies to Cache Valley virus can be provoked by day 76, to SV40 virus by day 90, to T4 phage by day 105, to bluetongue virus by day 122, and to lymphocytic choriomeningitis virus by day 140. The proportions of α/β and γ/δ T cells change as lambs mature. One month before birth 18% of blood T cells are γ/δ ⁺. By one month after birth they constitute 60% of blood T cells.

18.2.1.4

Piglet

The gestation period of the sow is about 115 days. B cells appear in the yolk sac at day 20, progress to the fetal liver by day 30, and progress to the bone marrow by day 45. The first SWC3⁺ leukocytes can be found in the yolk sac and liver on day 17. The thymus develops by 40 days postconception and is colonized by two waves of T cell progenitors beginning on day 38. γ/δ T cells appear first in the thymus and then in the peripheral blood. α/β T cells develop later, but their numbers grow rapidly so that they predominate late in gestation. The intestinal lymphoid tissues are devoid of T cells at birth. CD4⁺ T cells appear in the intestine at 2 weeks of age, and CD8⁺ T cells appear at 4 weeks. Their proliferation appears to be driven by the intestinal microflora. IgM⁺ B cells can be found in blood by gestation day 50. Fetal piglets can produce antibodies to parvoviruses at 58 days and can reject allografts at approximately the same time. Blood lymphocytes can respond to mitogens between 48 and 54 days. Natural killer (NK) cell activity does not develop until several weeks after birth, although cells with an NK phenotype can be identified in the fetal pig. B cells are the first lymphocytes to appear in peripheral blood. The number of circulating B cells rises significantly between 70 and 80 days gestation. The response to antigens in the fetus is essentially of the IgM type, but newborn and

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fetal piglets also produce a small immunoglobulin that may not have light chains. It is interesting to note that B cells can be found in the thymus of newborn pigs.

The molecular development of the antibody repertoire has been followed in the developing pig. Thus VDJ rearrangement is first seen in the fetal liver at day 30. However, the fetal piglet does not initially use all its *IGHV* or *IGHD* genes. Likewise, N-region addition does not occur before day 40, suggesting that the onset of terminal deoxynucleotidyltransferase activity occurs after that time. IgM, IgA, and IgG transcripts are present from 50 days in all major lymphoid organs. Piglets are thus born with relatively limited B cell diversity. B cell numbers increase for the first 4 weeks after birth, but their antigen-binding repertoire does not begin to expand until 4 to 6 weeks of age. Similar studies on rabbits have shown that the fetal immunoglobulin repertoire does not diversify until after birth and this appears to be stimulated by bacterial colonization of the gastrointestinal tract. CD4⁺ CD8⁺ T cells and CD2⁺ B cells do not appear in gnotobiotic animals and are absolutely dependent on contact of the immune system with live microorganisms.

18.2.1.5

Puppy

The gestation period of the bitch is about 60 days. The thymus differentiates between days 23 and 33, and fetal puppies can respond to phage fX174 by day 40. Blood lymphocytes can respond to phytohemagglutinin by 45 days postconception, and these cells can be detected in lymph nodes by 45 days and in the spleen by 55 days. The ability to reject allografts also develops around day 45, although rejection is slow at this stage, and fetal puppies may be made tolerant by intrauterine injection of an antigen before day 42. Thymic seeding of T cells to the secondary lymphoid organs and the development of humoral immune responses are therefore relatively late phenomena in the dog compared to the situation in the other domestic animals.

18.2.1.6

Kitten

Data on the ontogeny of the kitten are limited. B cells are seen in the fetal liver at 42 days postconception. Fetal kittens do make some IgG that can be detected in their serum before suckling, although this may be due to antibodies crossing the placenta.

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18.2.1.7

Chick

Stem cells arise in the yolk sac membrane and migrate to the thymus and bursa at 5 to 7 days incubation. These cells differentiate within the bursa, and follicles develop by day 12. Lymphocytes with surface IgM may be detected in the bursa by day 14, and antibodies to keyhole limpet hemocyanin and to sheep erythrocytes may be produced by 16 and 18 days incubation, respectively. Lymphocytes with surface IgY develop on day 21, around the time of hatching, whereas IgA-positive cells first appear in the intestine 3 to 7 days after hatching. Vaccination of 18-day embryonated eggs is commonly employed in the modern poultry industry. The major in ovo vaccine is against the Marek's disease herpesvirus, but others against Newcastle disease and coccidiosis are available and those against infectious bronchitis and infectious bursal disease are under development.

18.2.2

Development of Innate Immunity

The acquired immune system is entirely naïve at birth and as a result is very slow to respond to invaders. Innate immune responses are therefore critical for the survival in the first weeks of life. Newborns, however, produce a diverse array of antimicrobial molecules, including complement components at low levels, lectins such as the

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pentraxins and collectins, peptides such as the defensins, lactoferrin, and lysozyme. Surfactant proteins A and D as well as β -defensin 1 and toll-like receptor 4 (TLR4) are produced in the preterm lamb lung. As a result, invaders are opsonized and or killed relatively efficiently. TLRs are present and functional in the newborn. In the fetal pig, neutrophils at 90 days postconception are fully capable of phagocytosing bacteria such as *Staphylococcus aureus*. However, they are deficient in bactericidal activity, which only reaches adult levels 10 days later. Near birth, the phagocytic and bactericidal capacity of these neutrophils declines as a result of an increase in steroid levels. After birth, macrophages have depressed chemotactic responsiveness, and they are also able to support the growth of some viruses that macrophages from adult animals do not. Virucidal activity is gradually acquired, although this process appears to be under thymic influence. The macrophages from neonatally thymectomized mice do not acquire this resistance, perhaps as a result of a deficiency of interferon- γ (IFN- γ). The serum of newborn animals is also deficient in some complement components, resulting in a poor opsonic activity that is reflected in an increased susceptibility to infection. The neutrophils of newborn foals move relatively slowly as compared to those in their dams. However, phagocytosis and bactericidal activity are no different in fetal foal cells than in mare cells. Serum C3 increases rapidly after birth in newborn piglets and reaches adult levels by 14 days of age.

Interesting changes occur in the distribution of macrophages in the newborn pig. In newborn piglets there are very few pulmonary intravascular macrophages. However, during the first few days after birth, blood monocytes adhere to the pulmonary capillary endothelium and differentiate into macrophages. Thus in the newborn piglet 75% of particles are removed from the blood in the liver and spleen, but by 2 months of age 75% are removed in the lungs. The alveolar macrophages of newborn pigs have poor phagocytic activity, but this is effectively acquired by 7 days of age.

18.2.3 The Immune System and Intrauterine Infection

Although a fetus is not totally defenseless, it is less capable than an adult of combating infection. Its acquired immune system is not fully functional; as a result, some infections may be mild or unapparent in the mother but severe or lethal in the fetus. Examples include bluetongue, infectious bovine rhinotracheitis [bovine herpesvirus 1 (BHV-1)], bovine viral diarrhea (BVD), rubella in humans, and toxoplasmosis. Fetal infections commonly trigger an immune response as shown by lymphoid hyperplasia and elevated immunoglobulin levels. For this reason the presence of any immunoglobulins in the serum of a newborn, unsuckled animal suggests infection in utero.

In general, the response to these viruses is determined by the state of immunological development of the fetus. For example, if bluetongue virus vaccine, which is nonpathogenic for normal adult sheep, is given to pregnant ewes at 50 days postconception, it causes severe lesions in the nervous system of fetal lambs, including hydranencephaly and retinal dysplasia, whereas if it is given at 100 days postconception or to newborn lambs, only a mild inflammatory response is seen. Bluetongue vaccine virus given to fetal lambs between 50 and 70 days postconception may be isolated from lamb tissues for several weeks, but if given after 100 days, reisolation is not usually possible. Akabane virus acts in a similar fashion in lambs. If given before 30 to 36 days postconception it causes congenital deformities. If given to older fetuses it provokes antibody formation and is much less likely to cause malformations. Piglets that receive parvovirus before 55 days postconception will usually be aborted or stillborn. After 72 days, however, piglets will normally develop high levels of antibodies to the parvovirus and survive. Prenatal infection of calves with BHV-1 results in a fatal disease, in contrast to postnatal infections, which are relatively mild. The transition between these two types of infection occurs during the last month of pregnancy.

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The effects of the timing of viral infection are well seen with BVD virus (BVDV). Thus, if a newly pregnant cow is infected early in pregnancy (up to 50 days), she may abort. On the other hand, infections occurring between 50 and 120 days, before the fetus develops immune competence, lead to asymptomatic persistent infection because the calves develop tolerance to the virus ([Figure 18-2](#)). These calves are viremic yet, because of their tolerance, fail to make antibodies or T cells against the virus. Some of these calves may show minor neurological problems and failure to thrive, but many are clinically normal. If the cow is infected with BVDV between 100 and 180 days postconception, calves may be born with severe malformations involving the central nervous system and eye, as well as jaw defects, atrophy, and growth retardation. Vaccines containing modified live BVDV may have a similar effect if administered at the same time. Calves infected 150 to 180 days after gestation are usually clinically normal.

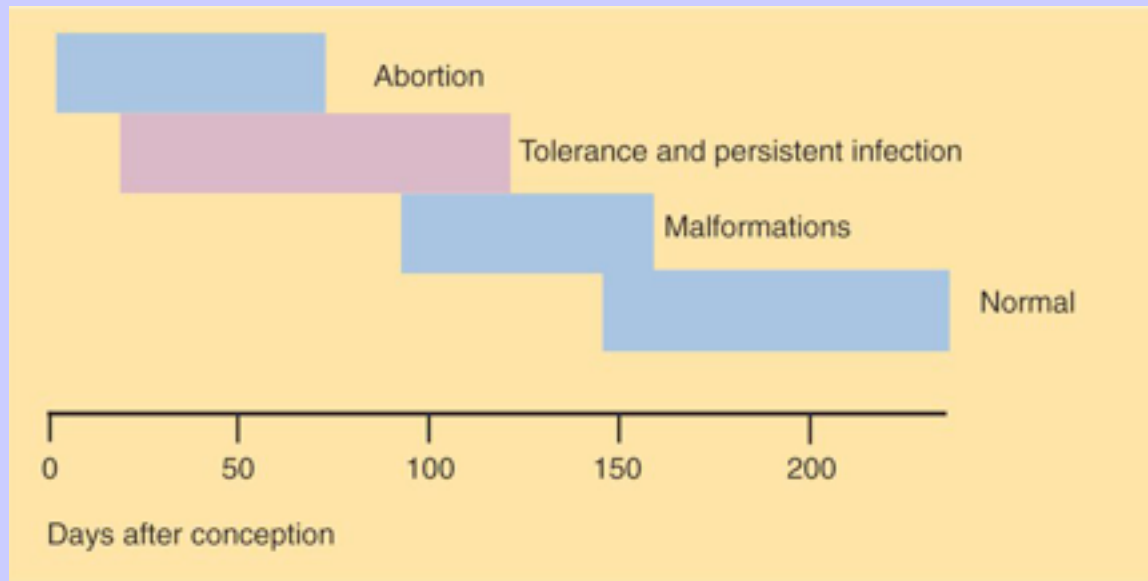
Since they are specifically tolerant to BVD, persistently infected calves shed large quantities of virus in their body secretions and excretions and so act as the major source of BVDV for other animals in a herd. The persistently infected calves may also produce neutralizing antibodies if immunized with a live BVD vaccine of a serotype different from that of the persistent virus. Despite this, the original virus will persist in these animals. These persistently infected calves grow slowly and often die of opportunistic infections such as pneumonia before reaching adulthood. (BVD has a tropism for lymphocytes and is immunosuppressive.) Their neutrophil phagocytic and bactericidal functions are also depressed.

BVD viruses occur in two distinct biotypes: cytopathic and noncytopathic. (The name derives from their behavior in cell culture, not their pathogenicity in animals.) Noncytopathic strains do not trigger type I interferon production and therefore can survive in calves and cause persistent infections. Cytopathic strains induce interferon production and cannot cause persistent infection. These cytopathic strains do, however, cause mucosal disease (MD), a severe enteric disease leading to profuse diarrhea and death ([Figure 18-3](#)). MD develops as a result of a mutation in a nonstructural viral gene that changes the BVDV biotype from noncytopathic to cytopathic while the animal fails to produce neutralizing antibodies or T cells. The cytopathic strain can spread between tolerant animals and lead to a severe MD outbreak. Both cytopathic and noncytopathic viruses can be isolated from these animals. Recombination may also occur between persistent noncytopathic strains and cytopathic strains administered in vaccines and lead to MD outbreaks. Although some of the lesions in MD are attributable to the direct pathogenic effects of the BVD virus, glomerulonephritis and other immune-complex-mediated lesions also develop. The reasons for this are unclear but may reflect superinfection or the production of nonneutralizing antibodies. Because persistently infected calves can reach adulthood and breed, it is possible for BVD infection to persist indefinitely within carrier animals and their progeny. Epidemiological studies suggest that between 0.4% and 1.7% of cattle in the United States are persistently infected in this way.

18.3 IMMUNE RESPONSE OF NEWBORN ANIMALS

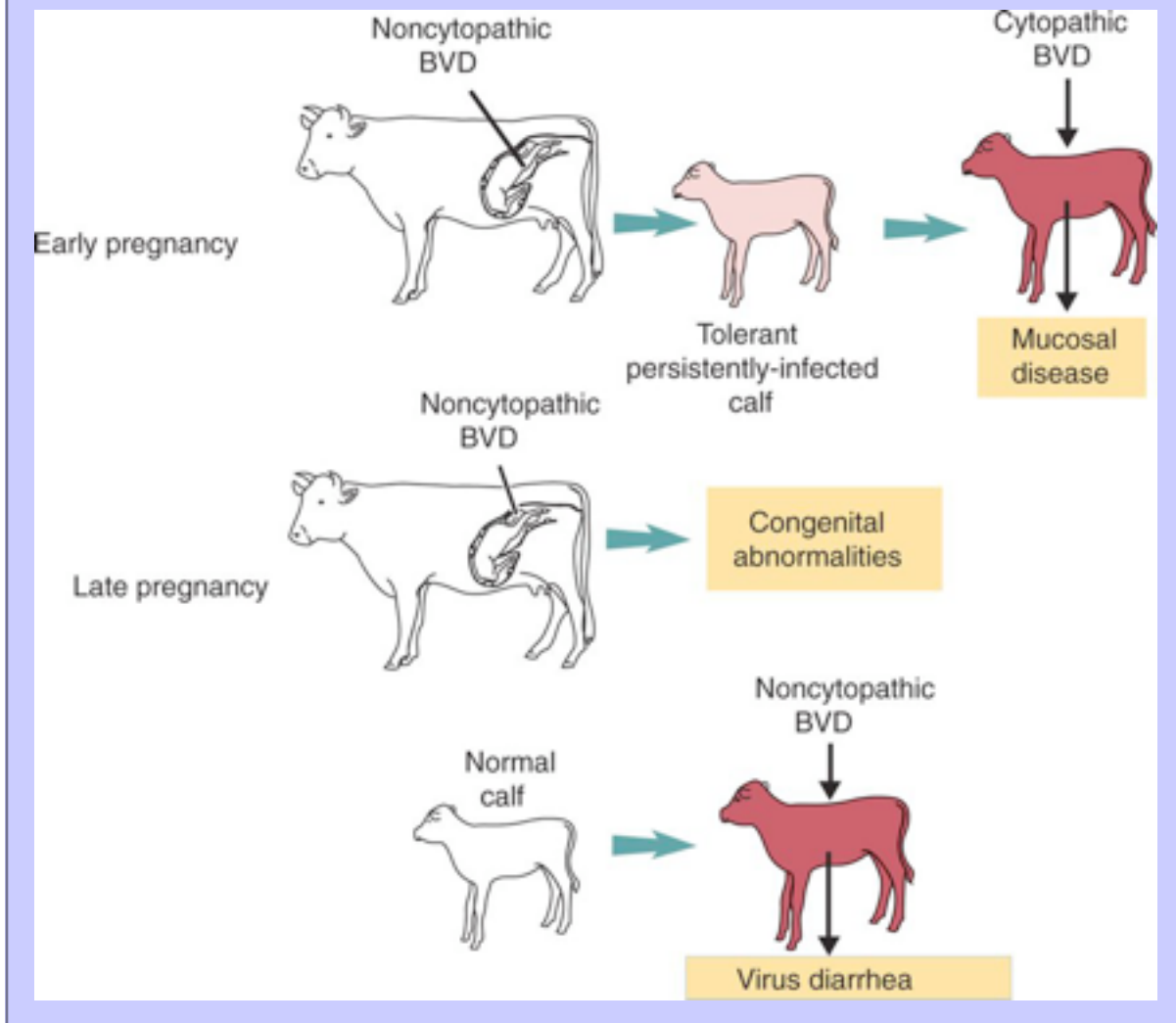
After developing in the sterile environment of the uterus, mammals are born into an environment rich in microorganisms. The young of domestic animals are

FIGURE 18-2 The effects of bovine viral diarrhea virus infection on development of the fetal calf depend on the timing of infection. As with adult animals, there is considerable individual variation in resistance to infection. Persistently infected calves may show minor neurological problems or failure to thrive.



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FIGURE 18-3 The relationship of mucosal disease (MD) to persistent infection with bovine viral diarrhea (BVD) virus in tolerant cattle. Calves persistently infected with noncytopathic BVD virus and then superinfected with cytopathic BVD virus develop MD.



capable of mounting both innate and acquired immune responses at birth. However, any acquired immune response mounted by a newborn must be a primary response with a prolonged lag period and low concentrations of antibodies. Newborn animals also mount immune responses skewed toward Th2 rather than Th1 cells. Neonatal Th1 cells appear to be highly sensitive to apoptosis induced by interleukin-4 (IL-4) and IL-13. Some Th1 cytokines, such as IFN- γ , appear to cause placental damage; therefore this skewing is not accidental and is probably a result of hormonal influences during pregnancy. Over the first months of life, the immune responses usually revert to the balanced adult pattern.

It is well recognized that newborn foals are highly susceptible to organisms such as *Rhodococcus equi*. Mononuclear cells from newborn foals are unable to express the IFN- γ gene. However, IFN- γ production does

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increase steadily through the first six months of life to reach adult levels within a year. Thus newborn foals are unable to mount Th1 responses, a feature that likely accounts for their susceptibility to intracellular pathogens. It is also clear that not all newborn foals are equally capable of fighting infections. Foals that develop pneumonia caused by *Rhodococcus equi* within a few weeks of birth were born with fewer leukocytes, fewer segmented neutrophils, and a much lower proportion of CD4⁺ T cells and a lower CD4 : CD8 ratio than normal.

The proper development of the newborn immune system depends in large part on exposure to intestinal microflora (see [Chapter 10](#)). “Germ-free” animals of some species may fail to develop gut-associated lymphoid tissues. The commensal flora produces a mixture of pathogen-associated molecular patterns and polysaccharides. Some are taken up by host dendritic cells and presented to CD4⁺ T cells. The T cells are then activated in a polyclonal fashion. Additionally a diversity of signals is received through TLRs. These signals collectively promote the complete functional development of the immune system. However, unless additional immunological assistance is provided, organisms that present little threat to an adult may kill newborn animals. This immunological assistance is provided by antibodies transferred from the mother to her offspring through colostrum. Maternal lymphocytes may also be transferred to the fetus through the placenta or to newborn animals through colostrum.

18.4 **TRANSFER OF IMMUNITY FROM MOTHER TO OFFSPRING**

The route by which maternal antibodies reach the fetus is determined by the structure of the placenta. In humans and other primates, the placenta is hemochorial: that is, the maternal blood is in direct contact with the trophoblast. This type of placenta allows maternal IgG but not IgM, IgA, or IgE to transfer to the fetus. Maternal IgG thus enters the fetal bloodstream and the newborn human infant has circulating IgG levels comparable to those of its mother.

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Dogs and cats have an endotheliochorial placenta in which the chorionic epithelium is in contact with the endothelium of the maternal capillaries. In these species 5% to 10% of IgG may be transferred from the mother to the puppy or kitten, but most must be obtained through colostrum.

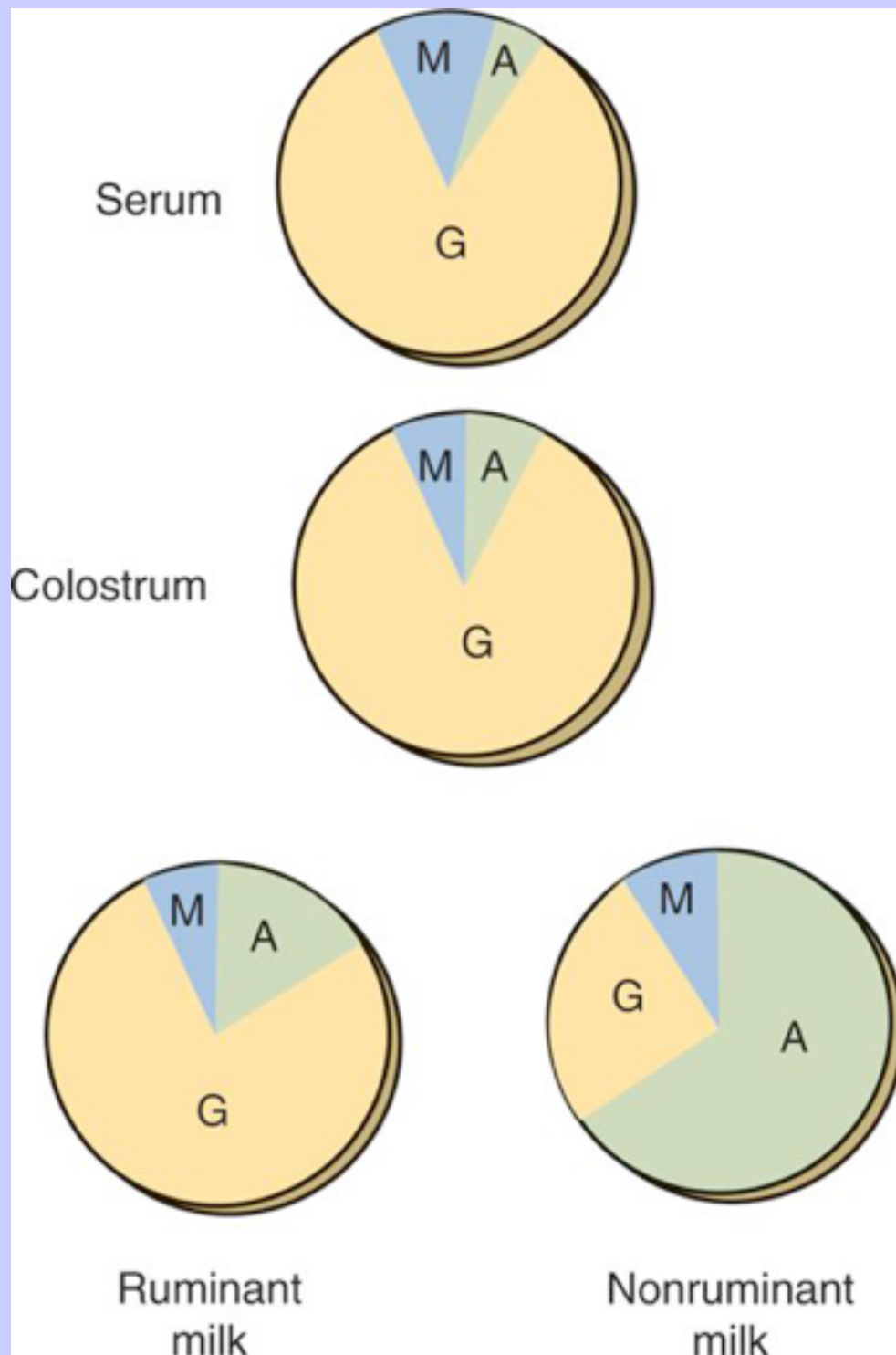
The placenta of ruminants is syndesmochorial—that is, the chorionic epithelium is in direct contact with uterine tissues—whereas the placenta of horses and pigs is epitheliochorial and the fetal chorionic epithelium is in contact with intact uterine epithelium. In animals with both these types of placenta, the transplacental passage of immunoglobulin molecules is totally prevented, and their newborn are entirely dependent on antibodies received through the colostrum.

18.4.1 **Secretion and Composition of Colostrum and Milk**

Colostrum contains the accumulated secretions of the mammary gland over the last few weeks of pregnancy together with proteins actively transferred from the bloodstream under the influence of estrogens and progesterone. Therefore it is rich in IgG and IgA but also contains some IgM and IgE ([Table 18-1](#)). The predominant immunoglobulin in the colostrum of most of the major domestic animals is IgG, which may account for 65% to 90% of its total antibody content; IgA and the other immunoglobulins are usually minor but significant components. As lactation progresses and colostrum changes to milk, differences among species emerge. In primates, IgA predominates in both colostrum and milk. In pigs and horses, IgG predominates in colostrum but its concentration drops rapidly as lactation proceeds, so that IgA comes to predominate in milk. In ruminants, IgG1 is the predominant immunoglobulin class in both milk and colostrum ([Figure 18-4](#)).

All of the IgG, most of the IgM, and about half of the IgA in bovine colostrum are derived by transfer from the cow's blood. In milk, by contrast, only 30% of the IgG and 10% of the IgA are so derived; the rest are

FIGURE 18-4 Relative concentrations of the major immunoglobulin classes in serum, colostrum, and the milk of ruminants and nonruminants.



produced locally by lymphoid tissue within the udder. Colostrum also contains secretory component both in the free form and bound to IgA. It is rich in cytokines as well. For example, bovine colostrum contains significant amounts of IL-1 β , IL-6, tumor necrosis factor- α , and IFN- γ . It has been suggested that these cytokines promote the development of the immune system in the young animal.

Table 18-1 Colostral and Milk Immunoglobulin Levels in Domestic Animals

Species	Fluid	Immunoglobulin (mg/dl)				
		IgA	IgM	IgG	IgG3	IgG6
Horse	Colostrum	500–1500	100–350	1500–5000	500–2500	50–150
	Milk	50–100	5–10	20–50	5–20	0
Cow	Colostrum	100–700	300–1300	2400–8000		
	Milk	10–50	10–20	50–750		
Ewe	Colostrum	100–700	400–1200	4000–6000		
	Milk	5–12	0–7	60–100		
Sow	Colostrum	950–1050	250–320	3000–7000		
Bitch	Colostrum	500–2200	14–57	120–300		
	Milk	110–620	10–54	1–3		
Queen	Colostrum	150–340	47–58	4400–3250		
	Milk	240–620	0	100–440		

18.4.2 Absorption of Colostrum

Young animals that suckle soon after birth take colostrum into their digestive tract. Thus naturally suckled calves ingest an average of 2 L of colostrum although individual calves may ingest as much as 6 L. In these young animals, the level of protease activity in the digestive tract is low and is further reduced by trypsin inhibitors in colostrum. Therefore colostral pro-teins are not degraded and used as food but instead reach the small intestine intact. Colostral immunoglobulins bind to special Fc receptors on the intestinal epithelial cells of newborns called FcRn. These are also expressed on mammary gland ductal and acinar cells and are probably involved in the active secre-tion of IgG into colostrum. FcRn is an MHC class Id molecule consisting of an a chain paired with β_2 -microglobulin. Once bound to FcRn, immunoglobulin molecules are endocytosed by intestinal epithelial cells and passed into the lacteals and possibly the intestinal capillaries. Eventually the absorbed immunoglobulin reaches the bloodstream, and newborn animals thus obtain a massive transfusion of maternal immunoglobulins.

Newborn mammals differ in the selectivity and duration of intestinal permeability. In the horse and pig, protein absorption is selective. IgG and IgM are preferentially absorbed, whereas IgA mainly remains in the intestine. In ruminants, absorption is unselective and all immunoglobulin classes are absorbed, although IgA is gradually excreted. Young pigs and probably other young animals have large amounts of free secretory component in their intestine. Colostral IgA and, to a lesser extent, IgM can bind this secretory component, which may then inhibit their absorption. IgE is also transferred in colostrum. For example, in sheep mean IgE levels in colostrum are

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three times higher than in ewe's serum. IgE is absent from presuckling lamb serum but rises to levels comparable to ewes by two days after birth. It declines to low levels by 30 days.

The duration of intestinal permeability varies between species and among immunoglobulin classes. In general, permeability is highest immediately after birth and declines after about 6 hours, perhaps because of the replacement of FcRn-bearing intestinal epithelial cells by cells that do not express the receptor. As a rule, absorption of all immunoglobulin classes will have dropped to a very low level after approximately 24 hours. Feeding colostrum tends to hasten this closure, whereas a delay in feeding results in a slight delay in closure (up to 33 hours). The presence of the mother may be associated with increased immunoglobulin absorption. Thus calves fed measured amounts of colostrum in the presence of the mother will absorb more immunoglobulins than calves fed the same amount in her absence. In laboratory studies where measured amounts of colostrum are fed, there is a great variation (25% to 35%) in the quantity of immunoglobulins absorbed. Management should ensure that foals or calves ingest at least 1 L of colostrum within 6 hours of birth. In piglets, the ability to absorb immunoglobulins may be retained for up to 4 days if milk products are withheld.

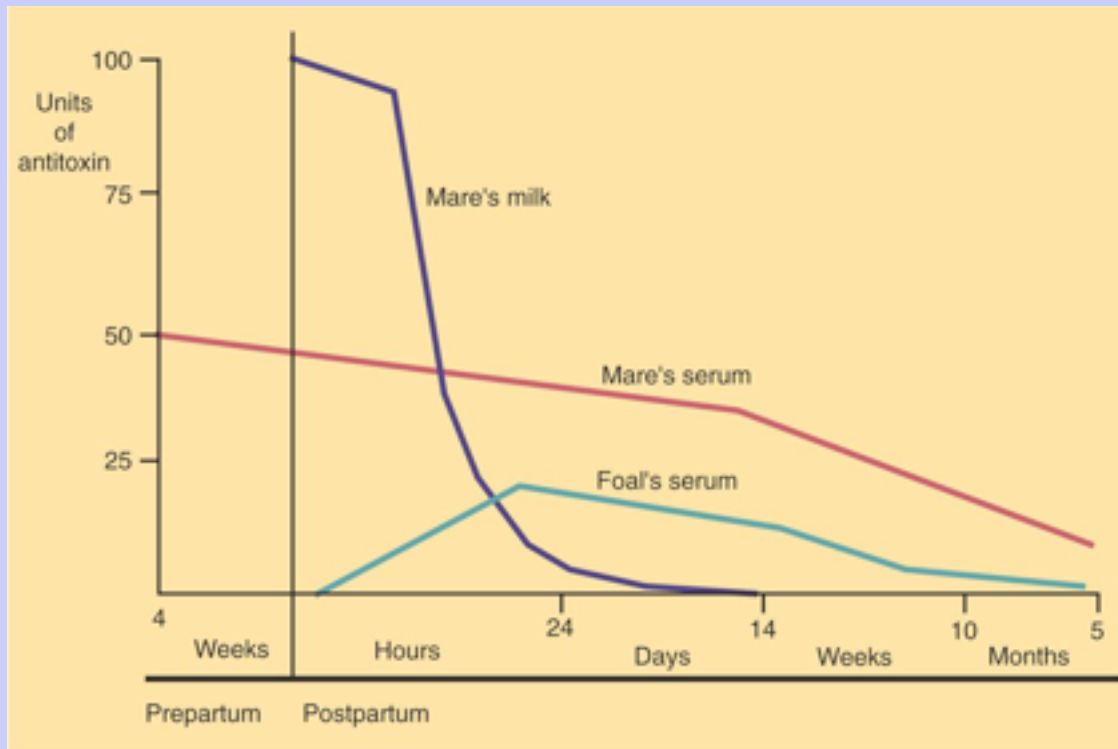
Unsuckled animals normally have very low levels of immunoglobulins in their serum. The successful absorption of colostral immunoglobulins immediately supplies them with serum IgG at a level approaching that found in adults ([Figure 18-5](#)). Peak serum immunoglobulin levels are normally reached between 12 and 24 hours after birth. After absorption ceases, these passively acquired antibodies decline through normal metabolic processes. The rate of decline differs among immunoglobulin classes, and the time taken to decline to unprotective levels depends on their initial concentration.

As intestinal absorption is taking place, a simultaneous proteinuria may occur. This is due to intestinal absorption of proteins such as β -lactoglobulin that are sufficiently small to be excreted in the urine. In addition, the glomeruli of newborn animals are permeable to macromolecules. Thus the urine of neonatal ruminants contains intact immunoglobulin molecules. This proteinuria ceases spontaneously with the termination of intestinal absorption. In another example, urine from puppies collected 24 hours after birth contains relatively large amounts of IgG, IgM, and IgA. The amount excreted declines over time so that IgM is undetectable by 14 days although there may still be significant amounts of IgG and IgA present. It is believed that the immunoglobulins enter the urine because glomerular filtration is insufficient. Over the first two weeks of life, the puppy's glomeruli mature and acquire the ability to filter macromolecules.

The secretions of the mammary gland gradually change from colostrum to milk. Ruminant milk is rich in IgG1 and IgA. Nonruminant milk is rich in IgA. For the first few weeks in life, while protease activity is low, these immunoglobulins can be found throughout the intestine and in the feces of young animals. As the digestive ability of the intestine increases, eventually only secretory IgA molecules remain intact. The amount of IgA in the intestine can be large: For instance,

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FIGURE 18-5 *Clostridium perfringens* antitoxin levels in serum, colostrum, and milk of six pony mares and in the serum of their foals from birth to 5 months. (After Jeffcott LB: *J Comp Pathol* 84:96, 1974.)



a 3-week-old piglet may receive 1.6 γ daily from sow's milk.

The IgG transferred through a mother's colostrum represents the consequences of her history of antigen exposure, B cell responses, and somatic mutation. This maternal IgG in effect represents the immunological experiences of the mother. Maternal antibodies act on the immune system of the newborn during a critical imprinting period and appear to exert a life-long influence on the newborn's immune development. This influence may be stronger than some genetic pre-dispositions. Thus maternal antibodies can enhance the newborn immune responses to some antigens and suppress their responses to others. Thus they may determine Th1/Th2 polarization, and they may trigger autoimmunity in the newborn.

18.5 FAILURE OF PASSIVE TRANSFER

The absorption of IgG from colostrum is required for the protection of a newborn against septicemic disease. The continuous intake of IgA or IgG1 from milk is required for protection against enteric disease ([Figure 18-6](#)). Failure of these processes predisposes a young animal to infection.

There are three major reasons for failure of passive transfer through colostrum. First, the mother may produce insufficient or poor-quality colostrum (production failure). Second, there may be sufficient colostrum produced but

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inadequate intake by the newborn animal (ingestion failure). Third, there may be a failure of absorption from the intestine despite an adequate intake of colostrum (absorption failure).

18.5.1 Production Failure

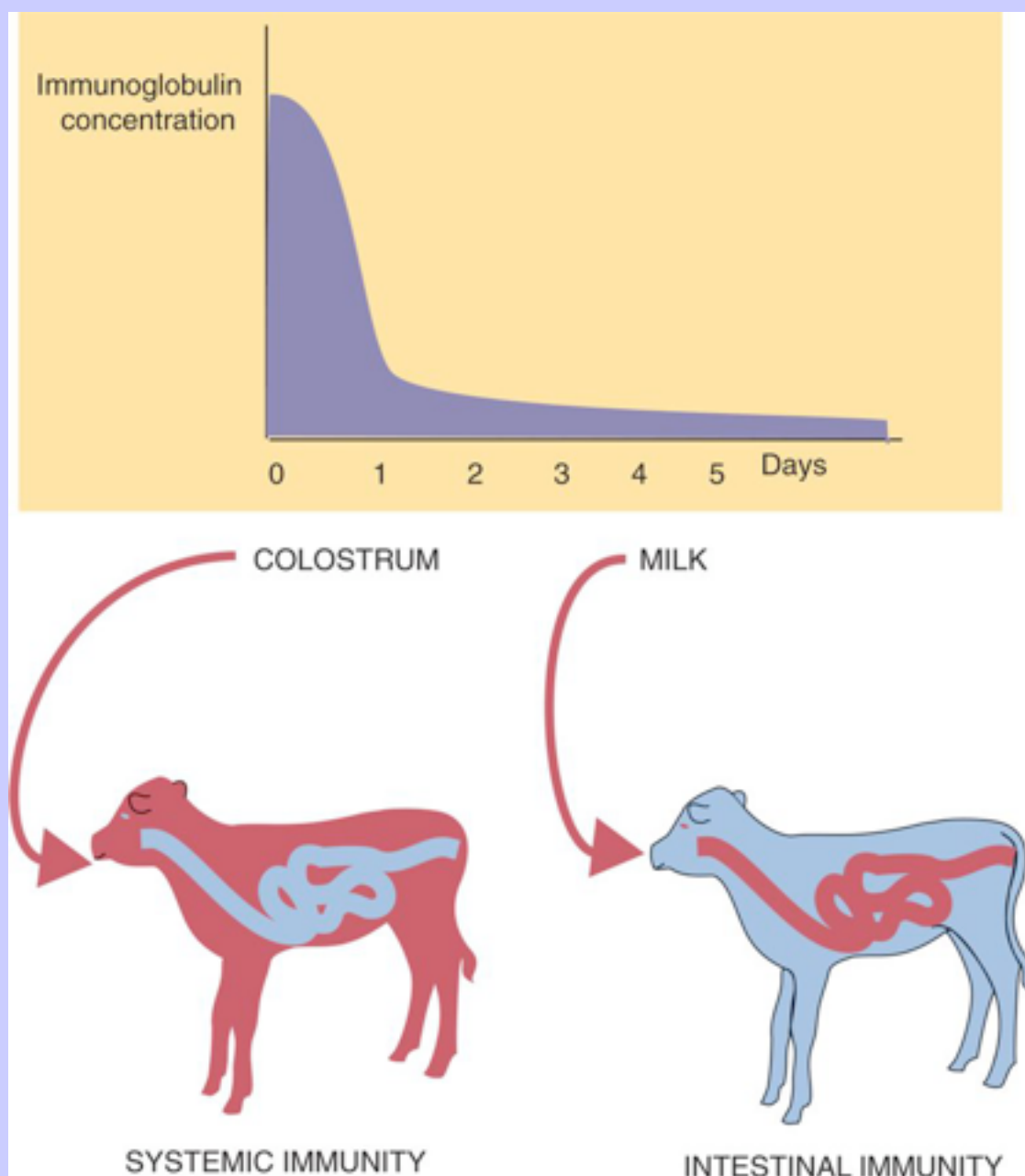
Since colostrum represents the accumulated secretions of the udder in late pregnancy, premature births may mean that insufficient colostrum has accumulated. Valuable colostrum may also be lost from animals as a result of premature lactation or excessive dripping before birth. Colostral IgG levels also vary between individuals, with up to 28% of mares producing low-quality colostrum. It is not possible to assess colostral quality simply by looking at it. Its IgG content should be assessed using a colostrometer (a modified hydrometer) to measure its specific gravity. This is normally in the range of 1.060 to 1.085, equivalent to an IgG concentration of 3000 to 8500 mg/dl. Colostrum with an IgG level of less than 3000 mg/dl may be inadequate to protect a foal, and supplemental high-quality colostrum may be required.

18.5.2 Ingestion Failure

In sheep or pigs, an inadequate intake may result from multiple births simply because the amount of colostrum produced does not rise in proportion to the number of newborn. It may be due to poor mothering, an important problem among young, inexperienced mothers. It also may be due to weakness in the

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FIGURE 18-6 Colostrum intake is required to protect young animals against septicemic disease. The prolonged intake of milk is necessary to ensure protection of the gastrointestinal tract against enteric infection.



newborn, to a poor suckling drive, or to physical problems such as damaged teats or jaw defects.

18.5.3 Absorption Failure

Failure of intestinal absorption is a major cause for concern in any species. It is especially important in horses not only because of the value of many foals but also because, even with good husbandry, about 25% of newborn foals fail to absorb sufficient quantities of immunoglobulins. Alpacas also appear to experience a disproportionate number of cases of failure of passive transfer. Foals require serum IgG concentrations of at least 800 mg/dl after receiving colostrum to ensure protection. Foals that have less IgG than this are at increased risk of infection. If their IgG level fails to reach 400 mg/dl, severe infections are assured ([Figure 18-7](#)).

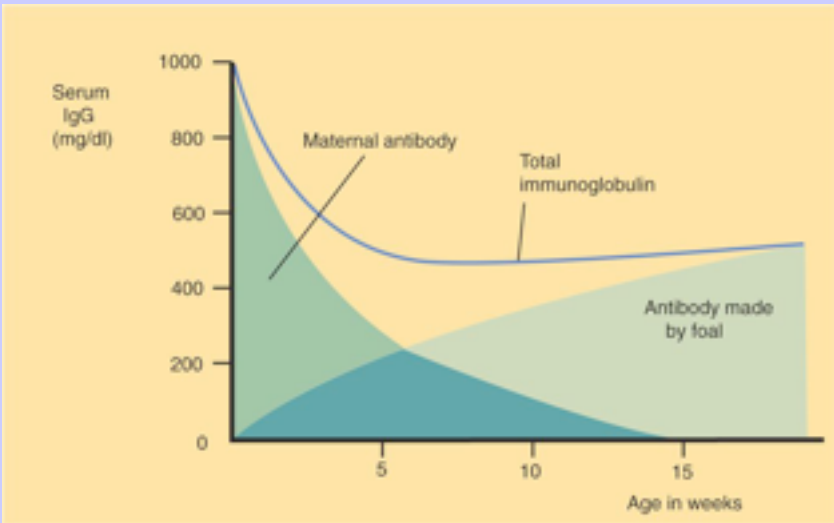
18.5.4 Diagnosis of Failure of Passive Transfer

The success of passive transfer cannot be evaluated in a foal until about 18 hours after birth, when antibody absorption is essentially complete. Several assays for serum immunoglobulins are available. The most rapid and economic procedure is the zinc sulfate turbidity test, which involves mixing a zinc sulfate solution with foal serum. Zinc sulfate makes globulins insoluble. In total failure of transfer, the reaction mixture remains clear. In sera with an IgG level of more than 400 mg/dl, the mixture becomes cloudy. As an alternative to visual inspection, the optical density of the mixtures can be read in a spectrophotometer and the IgG concentration read off a standard curve. Other similar techniques include precipitation by glutaraldehyde or by sodium sulfite.

Single radial immunodiffusion is a more accurate method because it is both quantitative and specific for IgG. As described in [Chapter 38](#), known standards are compared with the test serum by measuring the diameter of precipitation produced in agar gel containing an antiserum to equine IgG. A diagnosis of failure of passive transfer is made in foals if IgG levels are less than 400 mg/dl and partial failure of passive transfer if IgG levels are between 400 and 800 mg/dl. Unfortunately, radial immunodiffusion is expensive and slow. It takes 18 to 24 hours to give a result.

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FIGURE 18-7 Immunoglobulin levels in newborn serum during the first 15 weeks of life indicating the relative contributions of maternal antibody and antibody synthesized by the newborn animal.



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A third method of measuring IgG levels is to use a latex agglutination test. The latex particles are coated with antiequine IgG. In the presence of IgG they agglutinate. This test can be performed in about 10 minutes using either whole foal blood or serum. It appears to be reliable and rapid.

It is also possible to use a semiquantitative membrane-filter enzyme-linked immunosorbent assay (ELISA) test to measure IgG in a foal's serum. The color intensity of the reaction on the test filter is compared to color calibration spots. A variant technique uses a dipstick ELISA. Less satisfactory techniques include serum protein electrophoresis and refractometry.

18.5.5 Management of Failure of Passive Transfer

In foals, an IgG concentration higher than 800 mg/dl is preferred, but foals with immunoglobulin levels higher than 400 mg/dl will generally remain healthy and do not require treatment. About 75% of foals with IgG levels between 200 and 400 mg/dl will also remain healthy. However, they should be carefully watched and treated with antibiotics at the first signs of bacterial infection. Any foals with total failure of passive transfer or foals younger than 3 weeks with partial failure of passive transfer should be treated. Foals with plasma IgG concentrations less than 200 mg/dl, foals that have not nursed within 6 hours of birth, and foals that have received colostrum with IgG of less than 1000 mg/dl (specific gravity less than 1.050) should receive additional colostrum. Two to three liters of good-quality colostrum (IgG of more than 7000 mg/dl) should be given by bottle or nasogastric tube in three or four doses at hourly intervals. The colostrum must be free of antibodies to the foal's erythrocytes (see [Chapter 26](#)). Colostrum can be obtained from mares that have more than is needed for their own young. It can be stored frozen at -15°C to -20°C for up to one year. If stored colostrum is unavailable, fresh colostrum from primiparous mares can be used. If colostrum is not available, serum or plasma may be administered orally. A large volume (up to 9 L) may be required, since serum IgG is not well absorbed and its concentration is much less than that found in colostrum.

In foals that are older than 15 hours, oral absorption ceases and an intravenous plasma infusion must be given. Ideally the dose to be used can be calculated in order to attain an IgG level of at least 400 mg/dl. Frozen horse plasma is available commercially although this may not contain antibodies against local pathogens. Alternatively, the plasma may be obtained from local donors. Blood should be collected aseptically with heparin or sodium citrate. The plasma is collected after the erythrocytes settle and is stored frozen until used. The plasma must be prechecked for antierythrocyte antibody and must be free of bacterial contamination. The transfusion should be given slowly while the foal is monitored for untoward reactions. All foals receiving supplemental colostrum or plasma should have their IgG levels rechecked 12 to 24 hours later.

Considerations similar to those described above apply to failure of passive transfer in the calf. Calves with serum IgG concentrations of less than 1000 mg/dl at 24 to 48 hours of age have mortality rates more than twice than that of calves with higher IgG levels. Commercially available colostrum may be enriched with specific antibodies to protect the calf against potential pathogens such as K99 *Escherichia coli*, rotaviruses, and coronaviruses, the major causes of calf diarrhea.

Colostrum transfer of immunity is essential for the survival of young animals, but it may also cause disease. If a mother becomes immunized against the red cells of her fetus, colostrum antibodies may cause massive erythrocyte destruction in the newborn animal, a condition called hemolytic disease of the newborn (discussed at length in [Chapter 26](#)).

18.6 CELL-MEDIATED IMMUNITY IN MILK

Colostrum is full of lymphocytes. For example, sow colostrum contains between 1×10^5 and 1×10^6 lymphocytes/ml. Of these lymphocytes, 70% to 80% are T cells. The CD4/CD8 ratio is approximately 0.57, which is lower than the ratio in blood (1.4). Bovine colostrum also contains up to a million lymphocytes/ml, about half of which are T cells. There are usually very few lymphocytes in milk. Colostral lymphocytes may survive up to 36 hours in the intestine of newborn calves, and some penetrate the intestinal wall through the epithelium of Peyer's patches and reach the lacteal ducts or the mesenteric lymph nodes. Within 2 hours after receiving colostrum that contained labeled cells, piglets had maternal lymphocytes in their bloodstream. It is possible that cell-mediated immunity is transferred to newborn animals in this way. Piglets that had received these colostral cells showed enhanced responses to mitogens compared to control animals. Cell-containing and cell-free colostrum have been compared for their ability to protect calves against enteropathic *E. coli*. The calves receiving colostral cells excreted significantly fewer bacteria than the animals receiving cell-free colostrum. The concentration of IgA- and IgM-specific antibodies against *E. coli* in the serum of neonatal calves was higher in those that received colostral cells than in those that did not. The calves that received colostral cells had better responses to the mitogen concanavalin A and to foreign antigens such as sheep erythrocytes. The mechanisms of this protective effect are unclear.

Recently transfer of cell-mediated immunity by bovine milk lymphocytes has been demonstrated. Pregnant cows were vaccinated against BVDV. Blood lymphocytes from calves that received cell-free colostrum from these cows were unresponsive to BVDV antigen. In contrast, lymphocytes from calves that received colostrum-containing live cells showed enhanced responses to BVDV antigen at 1 and 2 days after colostral ingestion.

The lymphocytes of calves that received whole colostrum showed enhanced mitogenic responses to maternal and unrelated leukocytes after 24 hours. They also responded to the nonspecific stimulant Staphylococcus enterotoxin B. In contrast, the lymphocytes of calves that received acellular colostrum did not respond to either foreign leukocytes or to staphylococcal enterotoxin until 2 to 3 weeks after birth. Clearly, ingestion of maternal colostral leukocytes immediately after birth stimulates the development of the neonatal immune system.

18.7 DEVELOPMENT OF ACQUIRED IMMUNITY IN NEONATAL ANIMALS

18.7.1 Local Immunity

The intestinal lymphoid tissues of neonatal animals respond rapidly to an ingested antigen. For example, calves orally vaccinated with coronavirus vaccines at birth are resistant to virulent coronavirus within 3 to 9 days. Likewise, piglets vaccinated orally 3 days after birth with transmissible gastroenteritis virus vaccines develop neutralizing antibodies in the intestine 5 to 14 days later. Much of this early resistance is attributable to innate production of IFN- α/β , but there is an early intestinal IgM response that switches to IgA by 2 weeks. In the young animal, the IgA response appears earlier and reaches adult levels well before the other immunoglobulins. This rapid response of the unprimed intestinal tract is also seen in germ-free pigs. In these animals, antibody synthesis in the intestine can be detected by 4 days after infection with *E. coli*.

18.7.2 Systemic Immunity

The antibodies acquired by a young animal as a result of ingesting its mother's colostrum, maternal antibodies, will inhibit the ability of the newborn to mount its own immune response. As a result, very young animals are unable to respond to active immunization using vaccines. This inhibition is B cell-specific, and T cell responses

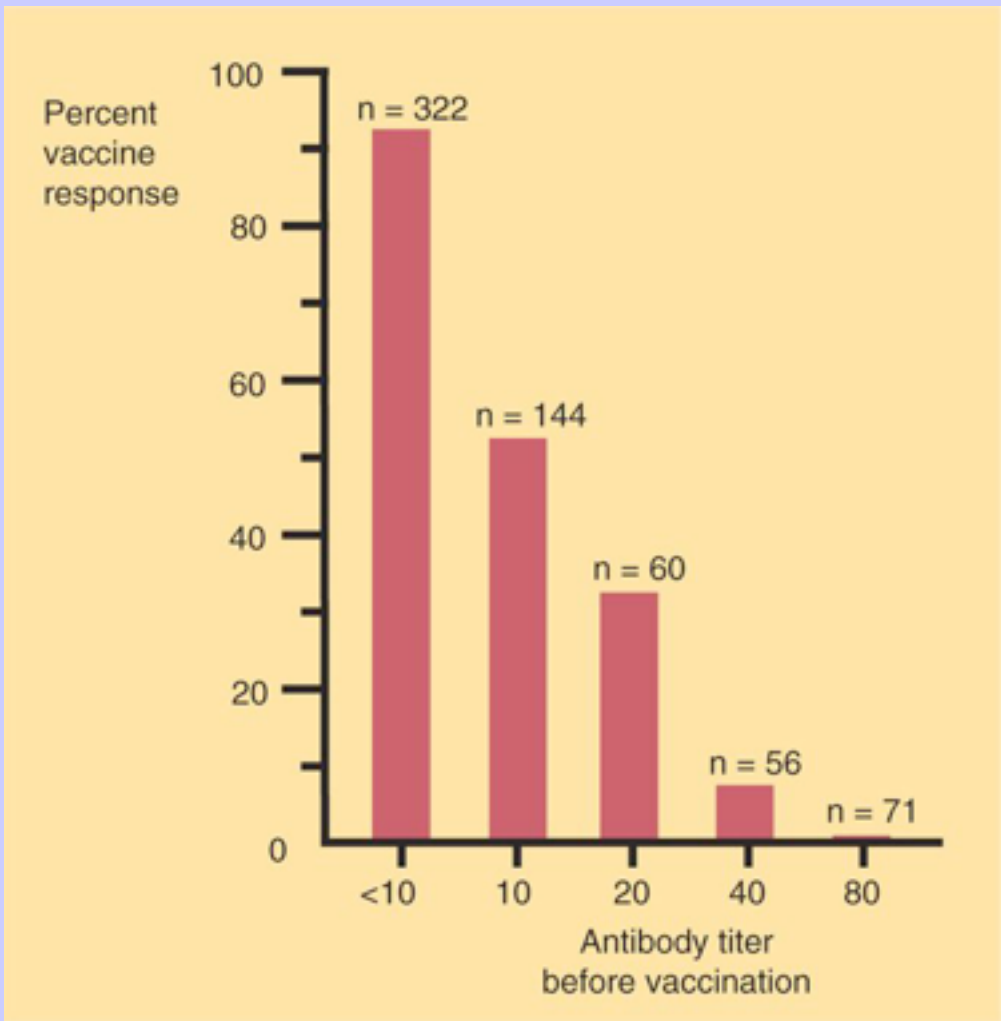
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are largely unaffected. It depends on the relative concentrations of maternal antibody and the dose of vaccine administered.

Several different mechanisms have been suggested as mediating this suppression. One of the simplest is the rapid neutralization of live viral vaccines by the maternal antibody. This would prevent viral replication and provide insufficient antigen to prime B cells. However, data from human infants and domestic mammals indicate that sufficient antigen is present to prime T cells. Likewise, this mechanism could not account for inhibition of the immune response to nonliving vaccines.

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FIGURE 18-8 Effect of the presence of maternal antibodies to canine parvovirus in 653 puppies on their response to a modified live parvovirus vaccine. The prevaccination antibody titer profoundly inhibits the response of the puppies to the vaccine. (From Carmichael LE: *Compend Contin Educ Pract Vet* 5:1043-1054, 1983.)



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A second proposed mechanism suggests that the inhibition results from antibodies binding to B cell Fc receptors and blocking BCR signaling ([Figure 18-8](#)). However, recent studies on mice whose Fc receptors have been deleted (FcR knockout mice) have shown that the ability of maternal antibodies to inhibit antibody responses is unaffected. This clearly cannot be the mechanism involved. Likewise, the suggestion that maternal antibodies bind antigen, which is then removed by Fc-dependent phagocytosis, cannot be correct.

A third suggested mechanism is that maternal antibodies simply mask the epitopes on vaccine antigens and so prevent their recognition by the animal's B cells. This suggestion is compatible with the selective inhibition of B cell responses, the lack of inhibition of T cell responses, and with the evidence that, at least in humans and mice, high doses of antigen can overcome maternal immunity. Thus for a given vaccine dose, an immune response can be elicited only when maternal antibody titers fall below a critical threshold.

In the absence of maternal antibodies, the newborn animal is able to make antibodies soon after birth. For example, if calves fail to suckle and are therefore hypogammaglobulinemic, they will begin to make their own antibodies by about 1 week of age. In calves that have suckled and thus possess maternal antibodies, antibody synthesis does not commence until about 4 weeks of age. Likewise, colostrum-deprived piglets respond well to pseudorabies virus by 2 days after birth, but if they have suckled, antibody production does not begin until 5 to 6 weeks after birth. Colostrum-deprived lambs synthesize IgG1 at 1 week and IgG2 by 3 to 4 weeks. In colostrum-fed lambs, however, IgG2 synthesis does not occur until 5 to 6 weeks of age.

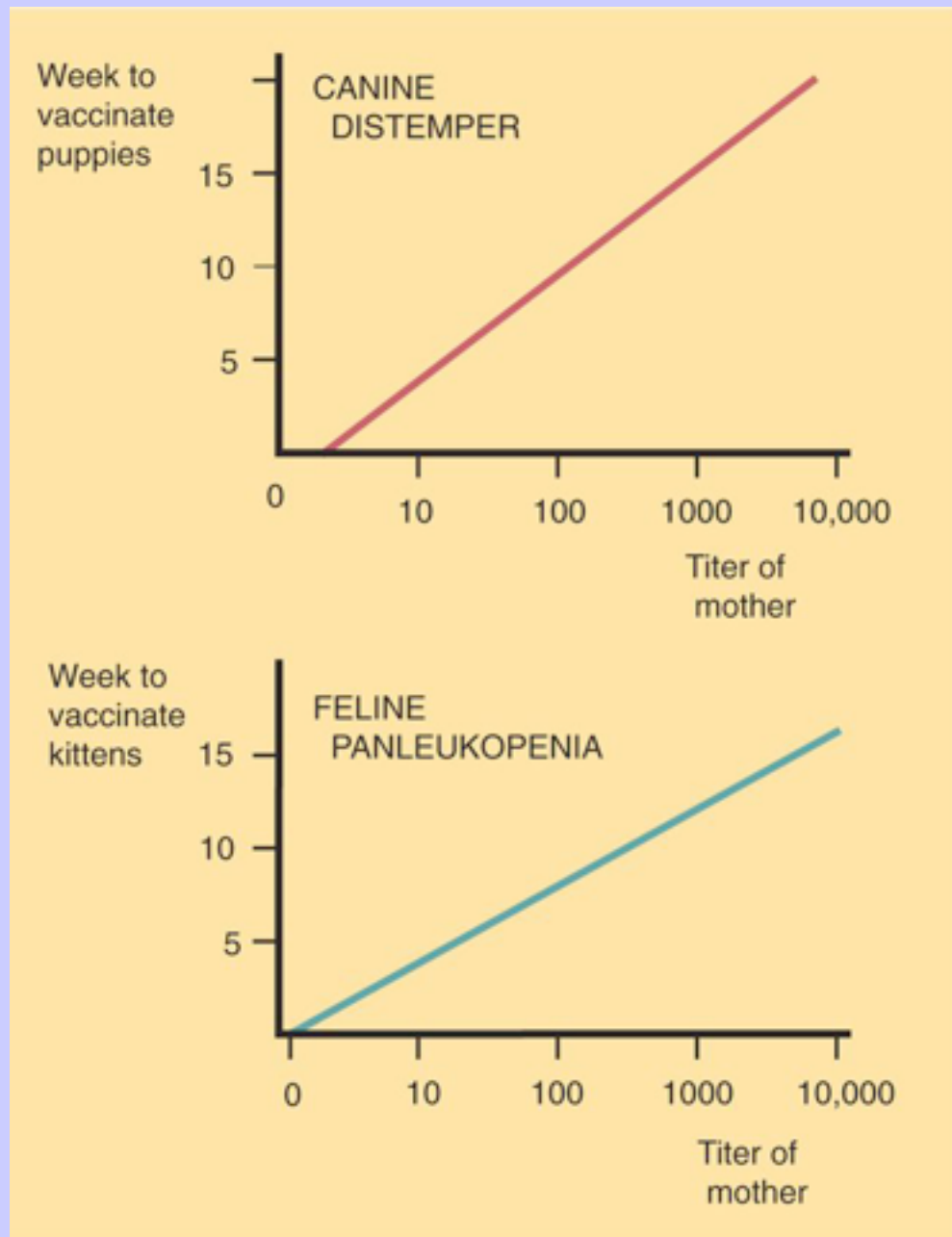
18.7.3 Vaccination of Young Animals

Because maternal antibodies inhibit neonatal immunoglobulin synthesis, they prevent the successful vaccination of young animals with conventional vaccines. This inhibition may persist for many months, its length depending on the amount of antibodies transferred and the half-life of the immunoglobulins involved. This problem can be illustrated using the example of vaccination of puppies against canine distemper.

Maternal antibodies, absorbed from the puppy's intestine, reach maximal levels in serum by 12 to 24 hours after birth. From then on their levels decline slowly through normal protein catabolism. The catabolic rate of proteins is exponential and is expressed as a half-life. Thus the half-life of antibodies to distemper and canine infectious hepatitis is 8.4 days, and the half-life of antibodies to feline panleukopenia is 9.5 days. Experience has shown that, *on average*, the level of maternal antibodies to distemper in puppies declines to insignificant levels by about 10 to 12 weeks, although this may range from 6 to 16 weeks. In a population of puppies, the proportion of nonimmune animals therefore increases gradually from a very few or none at birth, to almost all at 10 to 12 weeks. Consequently very few newborn puppies can be successfully vaccinated, but most can be protected by 10 to 12 weeks. Rarely, a puppy may be 15 or 16 weeks old before it can be successfully vaccinated. If canine distemper were not so common, it would be sufficient to delay vaccination until all puppies were about 12 weeks old, when success could be almost guaranteed. In practice, a delay of this type means that an increasing proportion of puppies, fully susceptible to disease, would be without immune protection—an unacceptable situation. Nor is it feasible to vaccinate all puppies repeatedly at short intervals from birth to 12 weeks, a procedure that would ensure almost complete protection. Therefore a compromise must be reached.

The earliest age to vaccinate a puppy or kitten with a reasonable expectation of success is between 6 and 9 weeks ([Box 18-2](#)). If the young animal is at unusually high risk of disease, it may be appropriate to begin vaccination somewhat earlier. Colostrum-deprived orphan pups may be vaccinated at 2 weeks of age. Normal puppies should be given distemper, two adenovirus, parvovirus, leptospirosis, and parainfluenza vaccines. In puppies a second dose should be given at

FIGURE 18-9 Nomographs showing the relationship between the antibody titer of the mother and the age at which to vaccinate her offspring with modified live virus vaccine. (Data from Scott FW, Csiza CK, Gillespie JH: *J Am Vet Med Assoc* 156:439-453, 1970 [feline panleukopenia]; and Baker JA, Robson DS, Gillespie JH, et al: *Cornell Vet* 49:158-167, 1959 [canine distemper].)



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9 to 12 weeks and a third at 13 to 16 weeks. Rabies vaccine may also be given at 16 weeks. In kittens an appropriate protocol would be to vaccinate against viral rhinotracheitis, calicivirus, and panleukopenia at 6 to 9 weeks, 9 to 12 weeks, and 12 to 14 weeks; feline leukemia vaccine can be given twice at 9 to 12 and 12 to 14 weeks; with rabies vaccine being given at 12 weeks. There are many similar alternative vaccination protocols, all aimed at conferring early protection while leaving as few puppies as possible unprotected ([Figure 18-9](#)).

18.7.3.1

Box 18-2 Measles Vaccine

An alternative approach to overcoming the problems caused by maternal immunity to canine distemper has been to use measles vaccine. Measles virus shares a major antigen, the F antigen, with canine distemper virus. The two viruses also possess distinctly different hemagglutinins (HA antigens). Generally, the presence of antibodies directed against both F and HA antigens is required for effective virus neutralization and complete protection, but antibodies to the individual antigens give partial protection. Thus maternal antidistemper HA antibodies cannot prevent measles infection of puppy cells. The measles virus F antigen may then prime the puppy's immune system. As a result, measles vaccine given to puppies at 6 weeks of age may induce a protective response in spite of the presence of a high level of antidistemper HA antibody.

Similar considerations apply when vaccinating large farm animals. The prime factor influencing the duration of maternal immunity is the level of antibodies in the mother's colostrum. Thus in foals maternal antibodies to tetanus toxin can last for 6 months and antibodies to equine arteritis virus for as long as 8 months. Antibodies to BVD may persist for up to 9 months in calves. The half-lives of maternal antibodies against equine influenza and equine arteritis virus antigens in the foal are 32 to 39 days. As in puppies, a young foal may have unprotective levels of maternal antibodies long before it can be vaccinated. Maternal antibodies, even at low levels, effectively block immune responses in young foals and calves, so that premature vaccination may be ineffective. The effectiveness of vaccines increases progressively after the first 6 months of life ([Figure 18-10](#)). A safe rule is that calves and foals should be vaccinated no earlier than 3 to 4 months of age followed by one or two revaccinations at 4-week intervals. The precise schedule will depend on the vaccine used and the species to be vaccinated. Animals vaccinated before 6 months of age should always be revaccinated at 6 months or after weaning to ensure protection.

Newly developed live recombinant vaccines such as canarypox-vectored distemper in dogs or influenza in horses appear to be able to effectively prime young animals in the face of significant maternal immunity. One possible reason for this is that mothers currently do not have antibodies against the canarypox vector, and it is possible that these vaccines may lose efficacy once a significant proportion of the maternal population is immune to canarypox. DNA vaccines against pseudorabies also appear to be effective in priming cell-mediated responses in the face of maternal immunity, whereas a DNA vaccine against bovine respiratory syncytial virus vaccine is not. Thus the ability of DNA vaccines to overcome maternal immunity varies between species and infections.

18.8

PASSIVE IMMUNITY IN THE CHICK

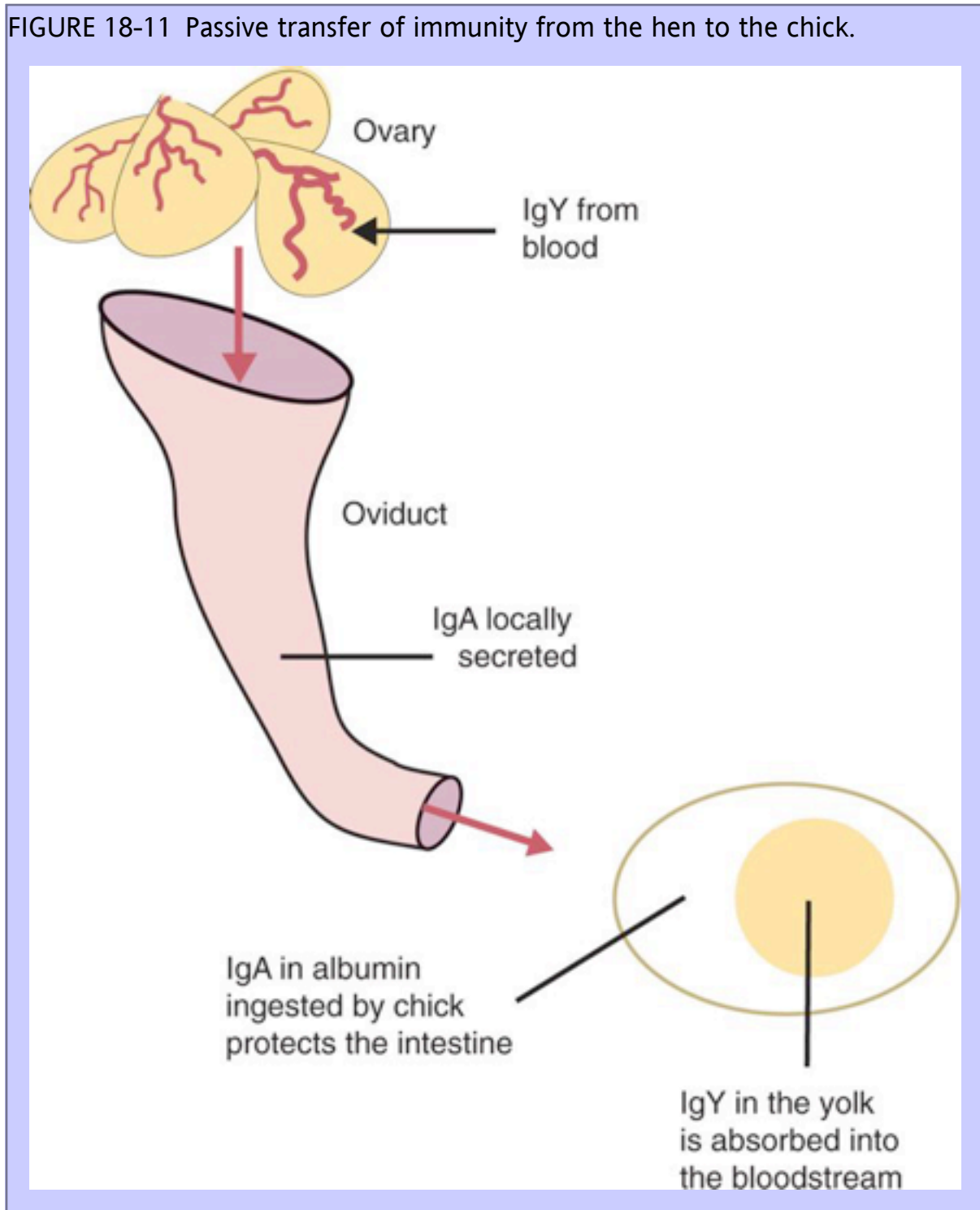
Newly hatched birds emerge from the sterile environment of the egg and, like mammals, require temporary immunological assistance. Serum immunoglobulins

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FIGURE 18-10 Effectiveness to two inactivated viral vaccines in calves between birth and 6 months of age. (Data courtesy Dr. R.J. Schultz.)



FIGURE 18-11 Passive transfer of immunity from the hen to the chick.



are actively transported from the hen's serum to the yolk while the egg is still in the ovary. (The immunoglobulin binds to an Fc receptor [FcRY].) In the fluid phase of egg yolk, IgY is therefore found at levels equal to or greater than those in hen serum. In addition, as the fertilized ovum passes down the oviduct, IgM and IgA from oviduct secretions are acquired with the albumin ([Figure 18-11](#)). As the chick embryo develops in ovo it absorbs the yolk

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IgY, which then appears in its circulation. At the same time, the IgM and IgA from the albumin diffuse into the amniotic fluid and are swallowed by the embryo. Thus when a chick hatches it possesses IgY in its serum and IgM and IgA in its intestine. The newly hatched chick does not absorb all its yolk sac antibodies until about 24 hours after hatching. These maternal antibodies effectively prevent successful vaccination until they disappear between 10 and 20 days after hatching.

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¹⁹ CHAPTER 19 Immunity at Body Surfaces

^{19.1} KEY POINTS

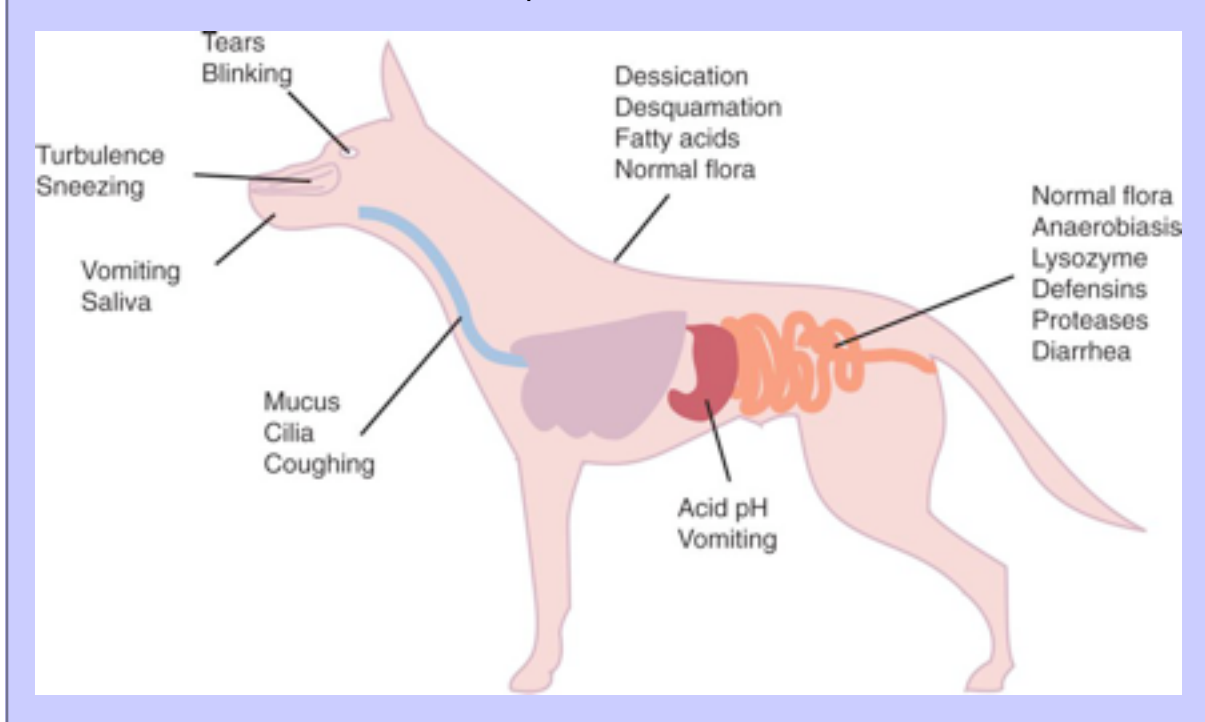
- Because it is essential to exclude microbial invaders, much of the body's lymphoid system is located on body surfaces such as the respiratory or digestive tract.
- The major surface defensive mechanisms seek to exclude invaders by preventing their penetration of surface epithelia.
- γ/δ T cells are T cells that are specialized for epithelial defenses.
- Immunoglobulin A (IgA), the major immunoglobulin on surfaces, prevents microbial adherence.
- IgE is a “back-up” surface defense. It triggers local acute inflammation in response to parasites that have succeeded in evading IgA.
- The normal intestine does not react to the gut microflora unless the organisms seek to invade the intestinal wall.
- The body responds in a limited fashion to antigens in food. However, unless allergies develop, this is rarely noticed.

Although mammals possess an extensive array of innate and acquired defense mechanisms within tissues, it is at their surfaces that invading microorganisms are first encountered and largely repelled or destroyed. Although the skin is the most obvious of these surfaces, it in fact represents only a small fraction of the area of the body exposed to the exterior. The areas of the mucous membranes of the intestine and respiratory tracts are at least 200 times larger. These surfaces are defended by both innate and acquired protective mechanisms.

^{19.2} INNATE PROTECTIVE MECHANISMS

One of the most important functions of the skin is to present a barrier to invading microorganisms ([Figure 19-1](#)). It presents a strong physical barrier supplemented by continuous desquamation, desiccation, and a low pH because of fatty acids in sebum. In addition, the skin carries a resident bacterial flora that excludes other bacteria and fungi. If this skin flora is disturbed, its protective properties are reduced, and microbial invasion may result. Thus skin infections tend to occur in areas such as the axilla or groin, where both pH and humidity are high. Similarly, animals forced to stand in water or mud show an increased frequency of foot infections as the skin becomes sodden, its structure breaks down, and its resident flora changes in response to alterations in the local environment. The importance of the resident

FIGURE 19-1 Some innate surface protection mechanisms.



flora is well seen in the intestine, where it is essential not only for the control of potential pathogens but also for the digestion of foods such as cellulose in the diet of herbivores. In addition, the natural development of the immune system in species such as the rabbit, sheep, and pig depends on the stimulation provided by intestinal microflora.

Because they have no bacterial flora, gnotobiotic (germ-free) pigs or mice have hypoplastic secondary lymphoid organs that do not develop germinal centers, and their immunoglobulin levels are only about 2% of normal. Thus the commensal bacteria in the gut induce maturation of the immune system. If the natural flora of the intestine is eliminated or its composition drastically altered (by aggressive antibiotic treatment, for example), an overgrowth of potential pathogens may occur, leading to severe colitis. The flora of the digestive tract normally acts competitively against potential invaders through mechanisms that supplement the other physical defenses of this system ([Figure 19-2](#)). Thus in the mouth, the flushing activity of saliva is complemented by the generation of peroxidases from streptococci. In single-stomached animals the gastric pH may be sufficiently low to have a bactericidal or virucidal effect, although this varies greatly among species and among meals. The dog, for instance, has a relatively low gastric pH relative to that of the pig. Similarly, the pH in the center of a mass of ingested food may not necessarily drop to low levels, and some foods such as milk are potent buffers.

Farther down the intestine, the resident bacterial flora keeps the pH and oxygen tension low. The intestinal flora is also influenced by the diet; for instance, the intestine of milk-fed animals is colonized largely by lactobacilli, which produce large quantities of bacteriostatic lactic and butyric acids. These acids inhibit colonization by potential pathogens such as *Escherichia coli*, so that young animals suckled naturally tend to have fewer digestive disturbances than animals weaned early in life. In the large intestine the bacterial flora mainly consists of strict anaerobes.

Specialized intestinal epithelial cells called Paneth cells secrete alpha defensins ([Figure 19-3](#)). These enteric defensins, known as cryptdins, accumulate within intestinal crypts and can achieve very high concentrations

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locally. They prevent bacteria from entering the crypt space and so protect enterocytes from invasion. In cattle, expression of cryptdin genes has been detected throughout the small intestine and colon. These bovine cryptdins are secreted as active molecules as opposed to the human and mouse molecules that are secreted as inactive precursors and activated by trypsin within the intestine. Parasitic or other intestinal infections may increase production of α and β defensins.

Lysozyme, the antibacterial and antiviral enzyme, is synthesized in the gastric mucosa and in macrophages within the intestinal mucosa. As a result, it is found in large quantities in intestinal fluid.

The intestinal microflora is essential for normal physiology. As a result mucosal immune responses to this microflora must be carefully regulated. This regulation is controlled by two cytokines, interleukin-2 (IL-2) and IL-10, which act through two distinct pathways. One involves toll-like receptors (TLRs), whereas the other does not. IL-10 negatively regulates the immune response to the intestinal microflora by inhibiting the TLR-MyD88 pathway. IL-2 inhibits commensal-induced mucosal inflammation by inhibiting TLR-independent pathways. Mice deficient in either IL-10 or IL-2 develop severe colitis.

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FIGURE 19-2 The role of the normal bacterial flora in excluding pathogens from body surfaces by competition. In the absence of a normal flora, the invading organisms face no competition and can readily colonize and invade surfaces.

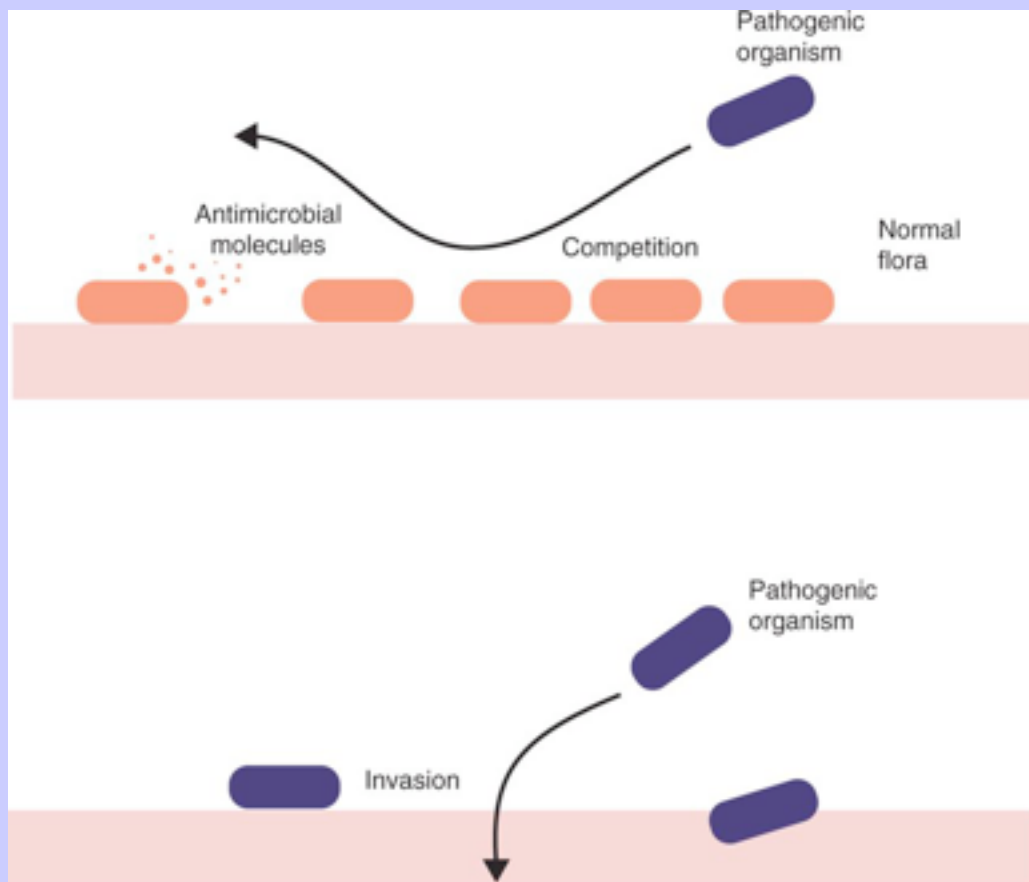
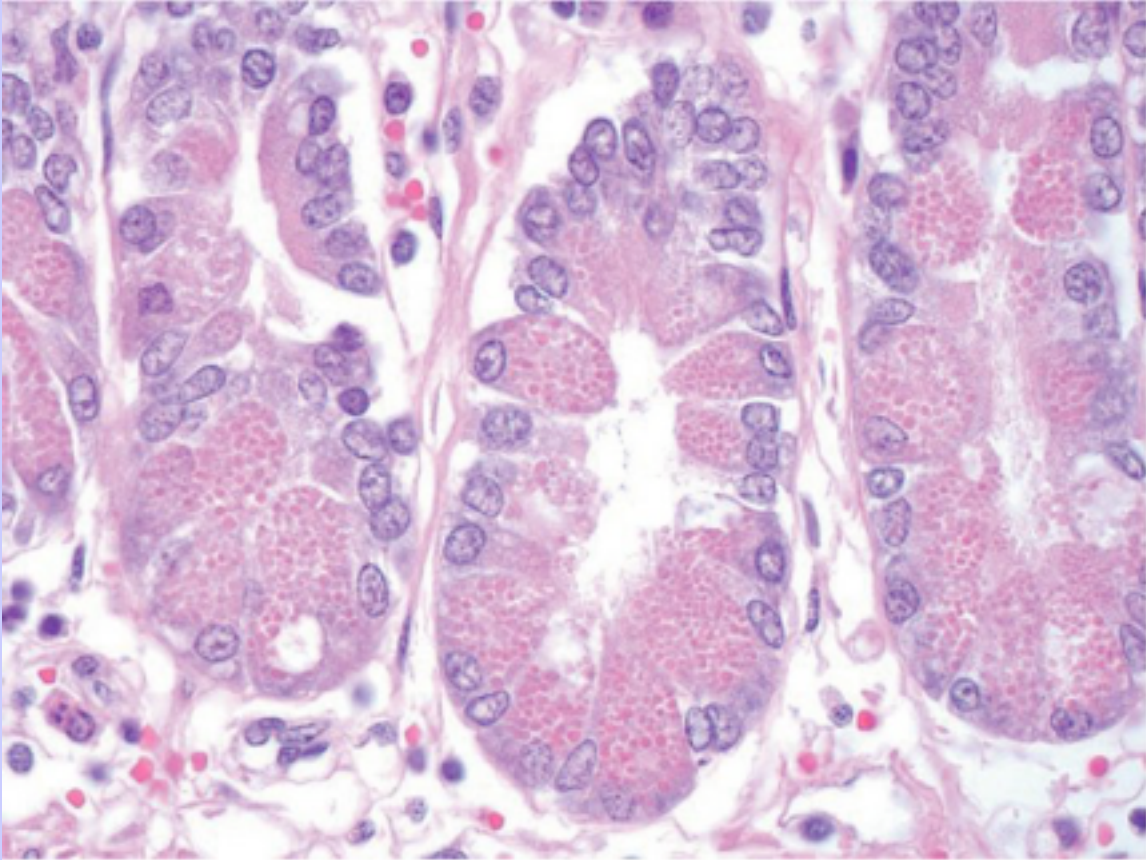
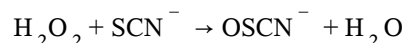


FIGURE 19-3 Paneth cells from the intestine of a horse. The cells are filled with large eosinophilic granules and are the major source of intestinal defensins. Original magnification $\times 60$. (Courtesy Dr. Brian Porter.)



In the urinary system, the flushing action and low pH of urine generally provide adequate protection; however, when urinary stasis occurs, urethritis resulting from the unhindered ascent of pathogenic bacteria is not uncommon. In adult animals, the vagina is populated almost exclusively by lactobacilli. The vagina is lined by a squamous epithelium composed of cells rich in glycogen. When these cells desquamate, they provide a substrate for the lactobacilli that, in turn, generate large quantities of lactic acid and reduce the pH to a level that protects the vagina against invasion by pathogenic bacteria and yeasts. Glycogen storage in the vaginal epithelial cells is stimulated by estrogens and thus occurs only in sexually mature individuals.

The mammary gland uses several different defense mechanisms. In a nonlactating animal, a keratin plug blocks the teat orifice and so excludes bacteria. In a lactating animal, the flushing action of the milk helps to prevent invasion by some potential pathogens, whereas milk itself contains many innate antibacterial substances (called lactenins). These antibacterial agents include complement, lysozyme, lactoferrin, and lactoperoxidase. Lactoferrin competes with bacteria for iron and makes it unavailable for their growth. It also enhances the neutrophil respiratory burst. Milk contains high concentrations of lactoperoxidase and thiocyanate (SCN^-) ions. In the presence of exogenous hydrogen peroxide, lactoperoxidase can oxidize the SCN^- to bacteriostatic products such as OSCN^- .



The hydrogen peroxide may be produced by bacteria such as streptococci or by the oxidation of ascorbic acid. 241

Some strains of streptococci are resistant to this bacteriostatic pathway, since they have an enzyme that reduces the 242

SCN^- . The phagocytic cells released into the mammary gland in response to irritation also contribute to antimicrobial resistance not only through their phagocytic efforts but also by providing additional lactoferrin, hydrogen peroxide, and lysosomal peroxidases. The binding of bovine lactoferrin to unencapsulated *Streptococcus agalactiae* can activate the classical complement pathway. The lactoferrin is apparently able to substitute for antibodies and activate C1q.

The respiratory tract differs from other body surfaces in that it is in intimate connection with the interior of the body yet it is required by its very nature to allow unhindered access of air to the alveoli. The system obviously requires a filter. Particles suspended in the air entering the respiratory tract are largely removed by turbulence that directs them onto its mucus-covered walls, where they adhere. The turbulence is caused by the conformation of the turbinate bones, the trachea, and the bronchi. This turbulence filter serves to remove particles as small as 5 μm before they reach the alveoli ([Figure 19-4](#)).

A blanket of sticky mucus produced by goblet cells covers the walls of the upper respiratory tract. The mucous gel absorbs soluble host defense molecules such as defensins, lysozyme, and immunoglobulin A (IgA) and thus has antimicrobial activity. This mucous layer is in continuous flow, being carried from the bronchioles up the bronchi and trachea by ciliary action or backward through the nasal cavity to the pharynx. Here the dirty mucus is swallowed and presumably digested in the intestinal tract. Particles smaller than 5 μm that can bypass this mucociliary escalator and reach the alveoli are phagocytosed by alveolar macrophages. Once these cells have successfully ingested particles, they migrate to the mucous escalator and are also carried to the pharynx and eliminated. Respiratory mucus may contain multiple antimicrobial molecules including the defensins and surfactant proteins (SPs) such as the collectins SP-A and SP-D.

19.3 LYMPHOID TISSUES

Because of the importance of preventing infection through mucosal surfaces, these surfaces contain large amounts of lymphoid tissue. These mucosal lymphoid tissues fall into two groups: sites where antigens are processed and immune responses are initiated (inductive sites) and sites where antibodies and cell-mediated responses are generated (effector sites).

19.3.1 Inductive Sites

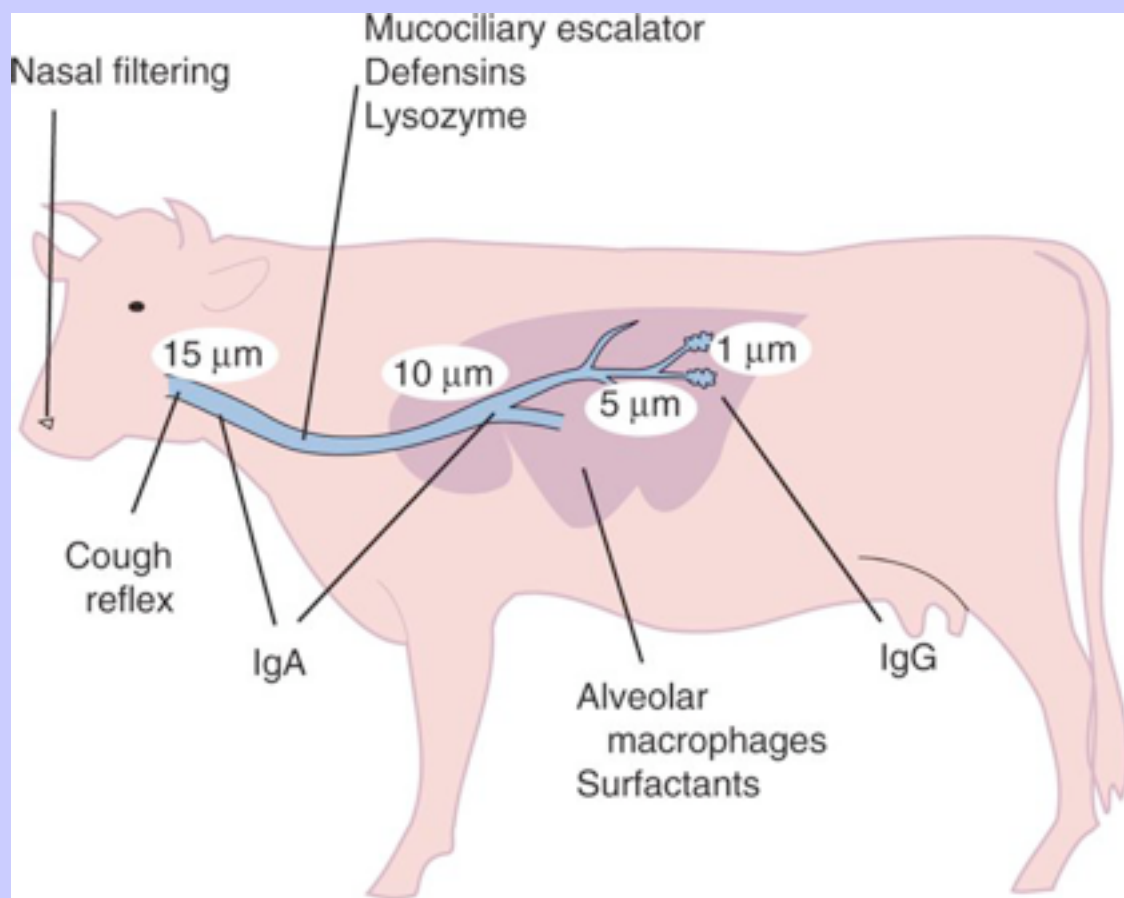
Mucosa-associated lymphoid tissues (MALT) possess all the components required to initiate immune responses: T cells, B cells, and dendritic cells. These include the lymphoid tissues in the eyelids and in the nasal mucosa, the tonsils and other lymphoid tissues in the pharynx, tongue, and palate (collectively called Waldeyer's ring), Peyer's patches, solitary lymphoid nodules, the appendix in the intestine, and numerous lymphoid nodules in the lung. These lymphoid tissues are known by their acronyms. Thus GALT (gut-associated lymphoid tissue) is the collective term for all the lymphoid nodules, Peyer's patches, and individual lymphocytes found in the intestinal walls. Similarly, BALT is the acronym used for the bronchus-associated lymphoid tissue in the lungs. These organized lymphoid tissues, unlike lymph nodes, do not encounter foreign antigens delivered through afferent lymph but sample it directly from the lumen.

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The tonsils are especially important in inducing immunity on mucosal surfaces. However, some organisms have developed mechanisms to overcome the defenses of the tonsils and use them as a portal of entry into the body. For example, pathogens such as bovine herpesvirus 1, *Mannheimia hemolytica*, *Streptococcus suis*, and *Mycobacterium tuberculosis* can persist indefinitely within the tonsils.

The surface of the intestine is covered by a layer of intestinal epithelial cells (enterocytes) that form intercellular tight junctions and which thus form an effective barrier to both microbes and macromolecules. (Molecules larger than about 2 kDa are excluded.) Obviously, some aggressive invasive bacteria can damage and penetrate enterocytes directly and so

FIGURE 19-4 Some of the innate mechanisms involved in the protection of the respiratory tract against infection and the influence of particle size on the site of deposition of particles within the respiratory tract. Note that only the smallest particles can penetrate deeply and gain access to the alveoli.



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trigger local inflammatory and immune responses. However, it is also clear that there is interplay between the antigen-processing cells in the intestinal wall and antigens in the lumen. Thus there are two other important routes by which organisms and macromolecules can penetrate the intact intestinal wall and be directed toward

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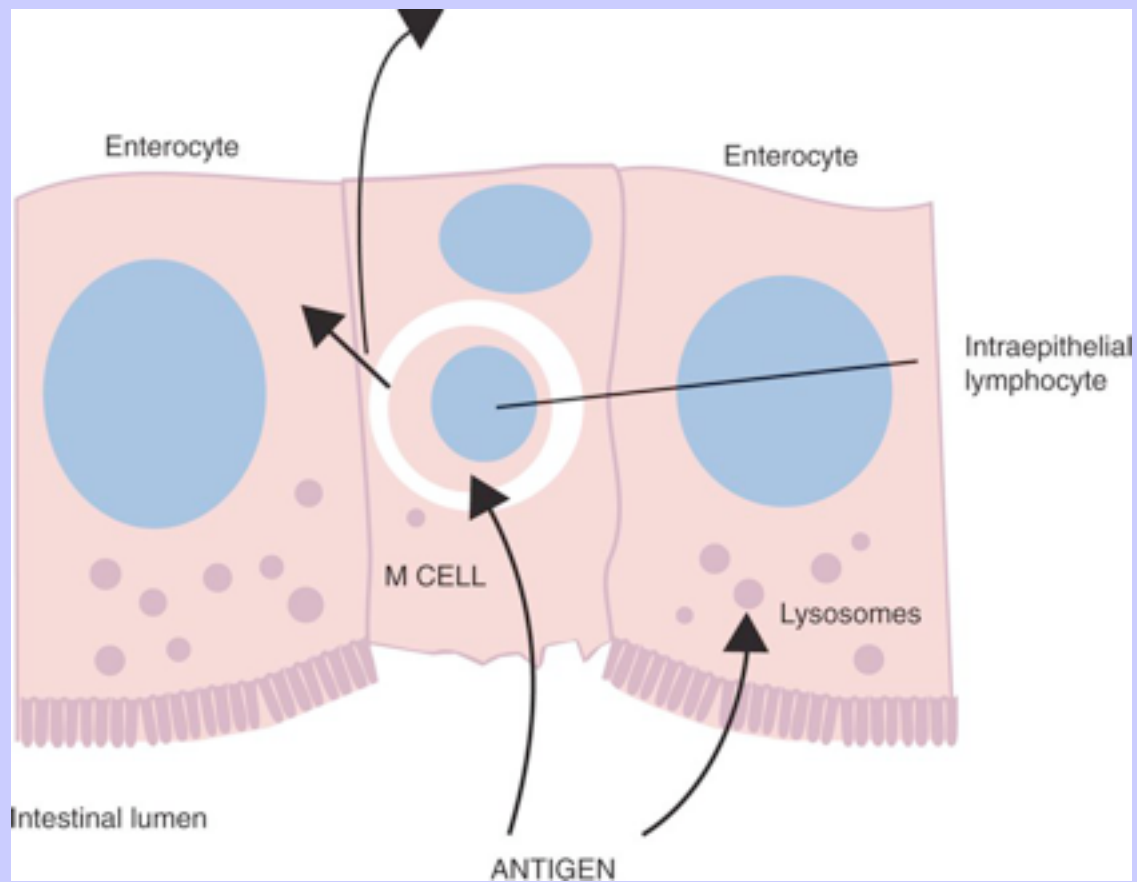
the intestinal lymphoid tissues. One route involves specialized cells (M cells) located directly over aggregates of lymphoid tissue or Peyer's patches; the other involves dendritic cells that reside in the submucosa but extend their cytoplasmic processes between the epithelial cells into the intestinal lumen. The tight junctions remain intact, but antigen samples can be transported within the dendritic cell cytoplasm. This route provides a mechanism whereby noninvasive bacteria and macromolecules can be sampled and presented to nearby T cells.

Peyer's patches are the largest of the mucosal lymphoid tissues. A newborn calf normally has about 100 Peyer's patches, and these may cover as much as half of the ileal surface. Collectively, therefore, the intestine contains more lymphocytes than the spleen. In ruminants and pigs there are two types of Peyer's patch that differ in location, structure, and functions. The ileocecal Peyer's patches are primary lymphoid organs, whereas the jejunal Peyer's patches are secondary lymphoid organs (see [Chapter 10, Figure 10-7](#)). In lambs, these ileocecal patches increase in size from birth to 6 months of age and then regress, leaving only a small scar in adults. In contrast, the jejunal patches persist throughout adult life and play a major role in intestinal defense. Both types of Peyer's patch consist of masses of lymphocytes arranged in follicles and covered with an epithelium that contains M cells (they have microfolds rather than microvilli on their surface) ([Figure 19-5](#)). M cells take up antigens from the intestinal lumen and present them directly to nearby lymphocytes. M cells may transport soluble macromolecules such as IgA, small particles, and even whole organisms. (Some pathogens such as salmonellae, *Yersinia*, *Listeria*, *M. tuberculosis*, and the reoviruses may take advantage of the M cells and use them to gain access to the body.) The proportion of M cells in the follicle-associated epithelium varies from 10% in humans and mice, to 50% in rabbits, and to 100% in the terminal ileum of pigs and calves.

19.3.2 Effector Sites

Although Peyer's patches contain large numbers of lymphocytes, most IgA is produced in diffuse lymphoid nodules and in isolated plasma cells located in the walls of the intestine, in bronchi, in salivary glands, and in the gallbladder. These cells constitute at least 80% of all plasma cells in the body. As a result, more IgA is produced every day than all other immunoglobulin classes combined. The presence of the gut microflora provides constant antigenic stimulus and

FIGURE 19-5 The role of M cells as antigen-processing cells in the intestinal wall. Antigen that enters enterocytes is usually rapidly degraded in lysosomes. Antigen that enters M cells is not degraded. It may be presented directly to intraepithelial lymphocytes within the M cell or, alternatively, permitted to pass along the intercellular space to the tissue fluid. From here, it will be carried to the draining lymph nodes.



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keeps intestinal lymphoid tissue in a constant state of activation.

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19.3.2.1

B Cells

The intestinal wall contains B cells that respond to antigen by dividing repeatedly. Some of these responding B cells migrate to regional lymph nodes and into intestinal lymphatics, from which they reach the thoracic duct and enter the bloodstream. These circulating IgA-positive B cells have an affinity for all body surfaces. As a result, they end up not only in the intestinal tract but also in the respiratory tract, urogenital tract, and mammary gland. Thus antigen priming at one location will permit antibodies to be synthesized and secondary responses to occur at locations remote from the priming site ([Figure 19-6](#)). The movement of IgA-positive B

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cells from the intestine to the mammary gland is especially important, since it provides a route by which intestinal immunity can be transferred to the newborn through milk. Oral administration of antigen to a pregnant animal will thus result in the appearance of IgA antibodies in its milk. In this way, antibodies directed against intestinal pathogens will flood the intestine of the newborn animal. T cells originating within the Peyer's patches also home specifically to the intestinal mucosa. This results from the use of specialized vascular addressins, molecules that determine lymphocyte migration patterns. For example, the mucosal vascular addressin cell adhesion molecule (MAdCAM-1) is expressed only on the high endothelial venules of Peyer's patches and on venules in intestinal lamina propria and the mammary gland. Its ligand is the lymphocyte integrin $\alpha 4/\beta 7$. As a result, B and T cells that express this integrin home preferentially to the intestine and the mammary gland.

19.3.2.2

T Cells

Both α/β and γ/δ T cells are found in the intestine, but in different locations. Thus in the intestine, α/β T cells are found in the lamina propria, whereas γ/δ T cells are found beneath and between enterocytes, where they are known as intraepithelial lymphocytes (IELs) ([Figure 19-7](#)). The number and location of these IELs suggest that they play a key role in the defense of the gastrointestinal tract, although there are species differences. Thus 5% of IEL in humans, 50% in mice, and up to 90% in ruminants carry γ/δ T cell antigen receptor (TCR). A high proportion of intestinal T cells are CD8⁺ (85% in humans, 77% in pigs, 24% in sheep). The CD8 molecules on these IELs consist of α/α homodimers in contrast to the CD8 α/β heterodimers found on conventional α/β T cells. Instead of binding to major histocompatibility complex (MHC) class II as in conventional responses, CD8 on IELs binds to an MHC class Ib molecule called TL (thymus-leukemia) antigen. TL antigen is expressed exclusively on enterocytes. The binding of T cells to TL molecules appears to suppress T cell function and thus prevents the killing of

FIGURE 19-6 When stimulated by antigen, immunoglobulin A (IgA)-producing B cells are produced in inductive sites, such as the Peyer's patches. They then leave the intestine and circulate in the bloodstream. They all eventually settle on other surfaces, such as the lung, the mammary gland, and other regions of the gastrointestinal tract-effector sites. It is this transfer of antibody-producing cells in the mammary gland that ensures that milk contains IgA antibodies directed against intestinal pathogens.

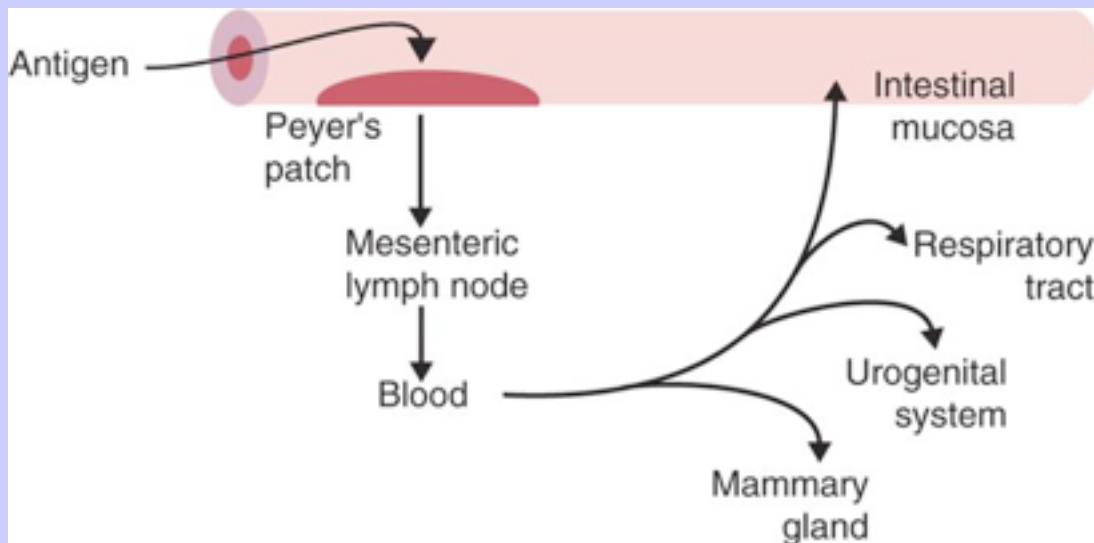
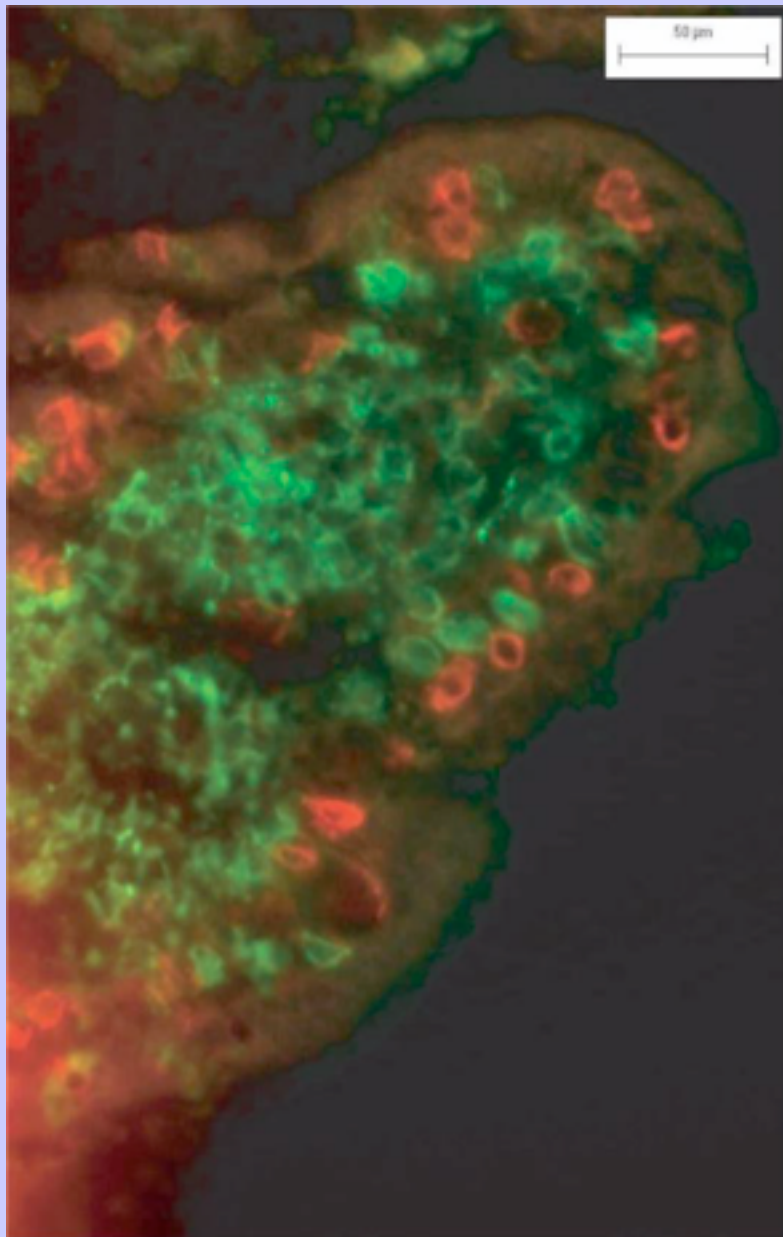


FIGURE 19-7 Double-color immunofluorescence showing a canine duodenal villous tip stained with monoclonal antibodies to α/β T cell antigen receptor (TCR) and γ/δ TCR. The α/β T cells are stained green and are located in the interior of the villus. The γ/δ T cells are stained red and are clearly located within the intestinal epithelium. (From German AJ, Hall EJ, Moore PF, et al: *J Comp Pathol* 121:249-263, 1999.)



enterocytes by the T cells. Conversely, enterocytes secrete molecules that promote γ/δ T cell survival. Bovine γ/δ T cells express CD36, a molecule previously thought to be restricted to myeloid and endothelial cells. It appears to be required for the efficient recognition of microbial lipoteichoic acids by these cells. It may well facilitate lipoteichoic acid recognition by TLR2 in much the same way as CD14 facilitates lipopolysaccharide recognition by TLR4.

IELs tend to use unusual Vg and Vd genes to form the TCR antigen-binding site. These genes are not expressed in other lymphoid organs, suggesting that the intraepithelial T cells are specialized for epithelial surveillance. The suggestion that γ/δ IEL may be a novel cell lineage is confirmed by the observation that they are found in neonatally thymectomized mice. They originate within the bone marrow and mature within cryptopatches, clusters of cells located just under the enterocytes. Cryptopatches each contain several hundred immature T cells with the surface markers c-kit (CD117, the receptor for SCF) and IL-7R (CD 127). IELs are MHC class II positive, and they may act as antigen-presenting cells on their own. IELs do not proliferate in response to conventional antigens. It is possible that their lack of proliferative response may be due to an absence of CD5 and CD28. Some γ/δ T cells have contrasuppressor activity and can prevent the development of oral tolerance within the intestinal lymphoid tissues (see [Chapter 17](#)). They may also regulate B cell IgA responses. Some have natural killer activity, whereas others are cytotoxic T cells that may attack parasites within the intestinal lumen. One unique property of these γ/δ T cells is that they can recognize antigens directly without prior processing. They secrete cytokines such as interferon- γ (IFN- γ) in response. The interferon can in turn stimulate macrophages and nearby enterocytes to secrete nitric oxide. This nitric oxide may serve a protective role in the intestinal mucosa.

Globule leukocytes are a subset of γ/δ T cells found in the cat and goat. They contain large eosinophilic cytoplasmic granules, but their nucleus resembles that of lymphocytes. Their function is unknown.

Ruminant γ/δ T cells recirculate continuously between epithelial surfaces such as the skin or intestinal epithelium and the bloodstream. In sheep, they are primarily located in skin near the basal layer of the epidermis and in the dermis close to hair follicles and sebaceous glands. They are uncommon in wool-covered skin but are present in large numbers in bare and hairy skin. In addition, they are found in the epithelium of the tongue, esophagus, trachea, and bladder.

19.4 ACQUIRED PROTECTIVE MECHANISMS

Both antibody- and cell-mediated immune processes protect body surfaces. The antibodies produced on mucosal surfaces include IgA, IgM, IgE, and IgG. Some of these, most notably IgA and possibly IgM, act by immune exclusion ([Figure 19-8](#)). The others, especially IgE and IgG, destroy antigen within the surface tissues by immune elimination.

19.4.1 Immune Exclusion

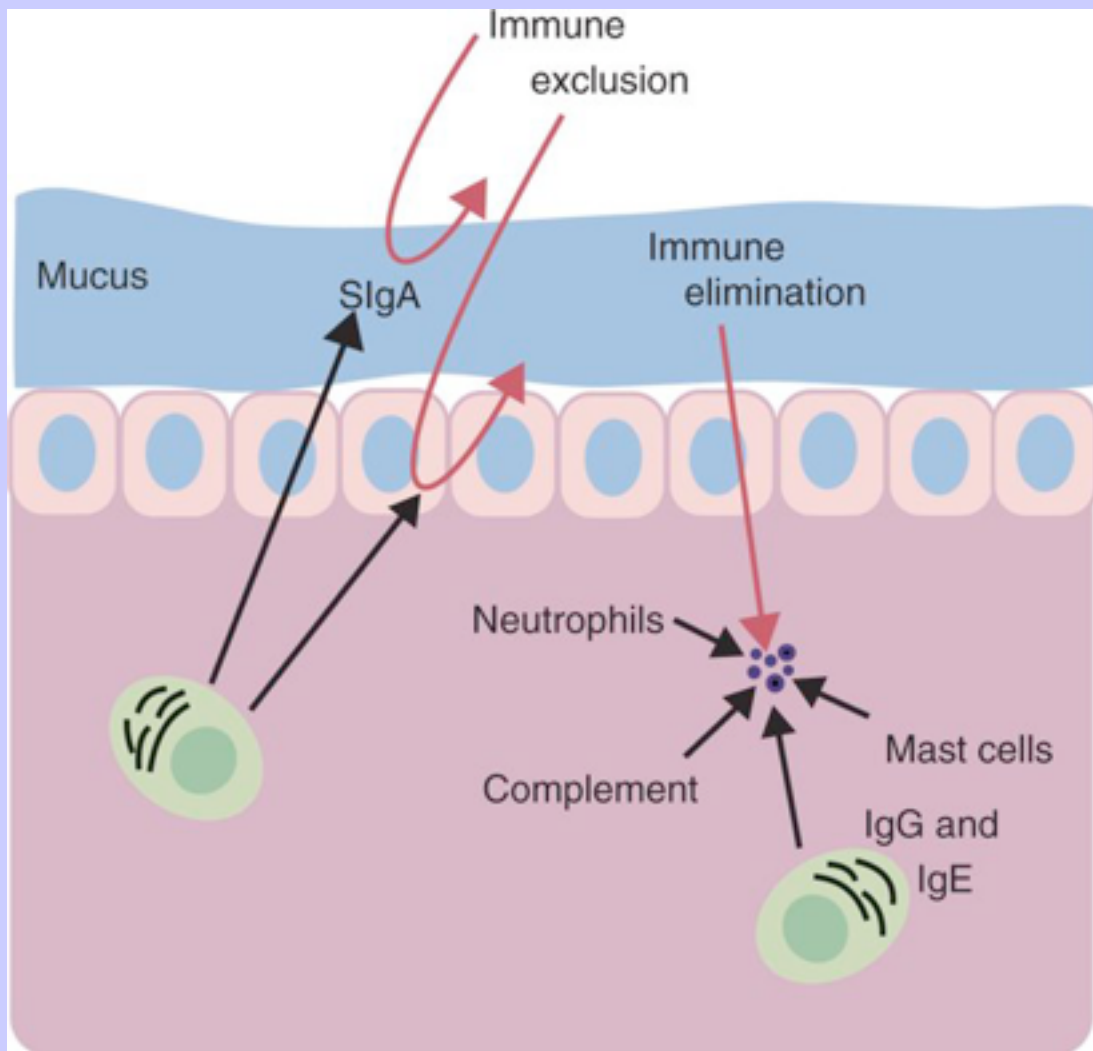
19.4.1.1 Immunoglobulin A

Immunoglobulin A predominates in surface secretions; it is found in significant amounts in saliva, intestinal fluid, nasal and tracheal secretions, tears, milk, colostrum, urine, and the secretions of the urogenital tract ([Figure 19-9](#)). IgA appears to have evolved specifically to protect body surfaces ([Table 19-1](#)). Thus in swine 90% of immunoglobulin-containing cells in the intestinal lamina propria contain IgA.

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Th2 cells are the predominant helper cells found in surface tissues. When appropriately stimulated, these Th2 cells secrete cytokines that result in the preferential production of IgA and IgE. Transforming growth factor- β (TGF- β) is the key cytokine that triggers this switch to IgA production ([Figure 19-10](#)), whereas IL-6 is essential for the terminal differentiation of IgA-producing plasma cells. Other Th2 cytokines, such as IL-4, IL-5, and IL-10, also promote these processes.

FIGURE 19-8 Two key defensive mechanisms are employed on mucosal surfaces. The most important is immune exclusion, an effect primarily mediated by immunoglobulin A (*IgA*). If antigens gain access to the mucosa, they are then destroyed by IgG- and IgE-mediated processes by immune elimination.



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The IgA monomer has a molecular weight of about 160 kDa and is a typical four-chain, Y-shaped molecule (see [Chapter 14](#), [Figure 14-6](#)). It is usually secreted as a dimer or larger polymer linked by a J chain. IgA has several extra cysteine residues in its heavy chains. As a result, the short interchain disulfide bonds compact the chains and shield vulnerable bonds from proteases.

IgA is synthesized and secreted by plasma cells in the intestinal submucosa, especially in the crypt region. This dimeric IgA binds to a glycoprotein receptor for polymeric immunoglobulins (pIgR) on the basal surface of enterocytes ([Figure 19-11](#)). Once bound, the receptor forms disulfide bonds with the Ca²⁺ domain of one of the IgA monomers. The complex of IgA and pIgR is then endocytosed and actively transported across the enterocyte. When it reaches the exterior surface, the endocytic vesicle fuses with the plasma membrane and exposes the IgA to the intestinal lumen. The extracellular domains of the pIgR are then cleaved by proteases so that the IgA, with the receptor peptide still attached (secretory IgA), is released into the lumen. The receptor peptide is called secretory component. The production, transport, and secretion of secretory component occur even in the absence of IgA so that free secretory component is found in high concentrations in intestinal secretions.

IgA is not bactericidal and does not activate the classical complement pathway. It can neutralize viruses, as well as some viral and bacterial enzymes, and it can act as an opsonin and function in some antibody-dependent cellular cytotoxicity (ADCC)

FIGURE 19-9 Typical immunoglobulin A (IgA) levels in bovine body fluids. In other species, milk and colostrum IgA concentrations may be considerably higher.

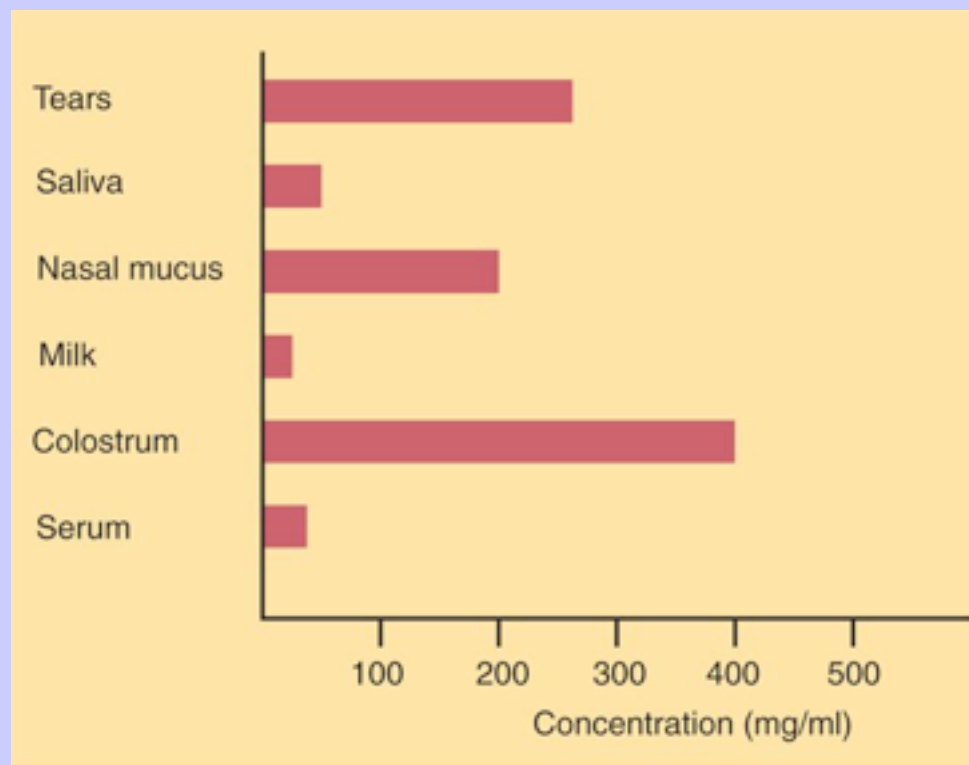


FIGURE 19-10 The control of immunoglobulin A (*IgA*) production. Transforming growth factor- β (*TGF- β*) is primarily responsible for the IgM-to-IgA switch. Terminal differentiation of IgA-producing plasma cells is mainly mediated by interleukin-6 (*IL-6*). Other cytokines are also important in the process.

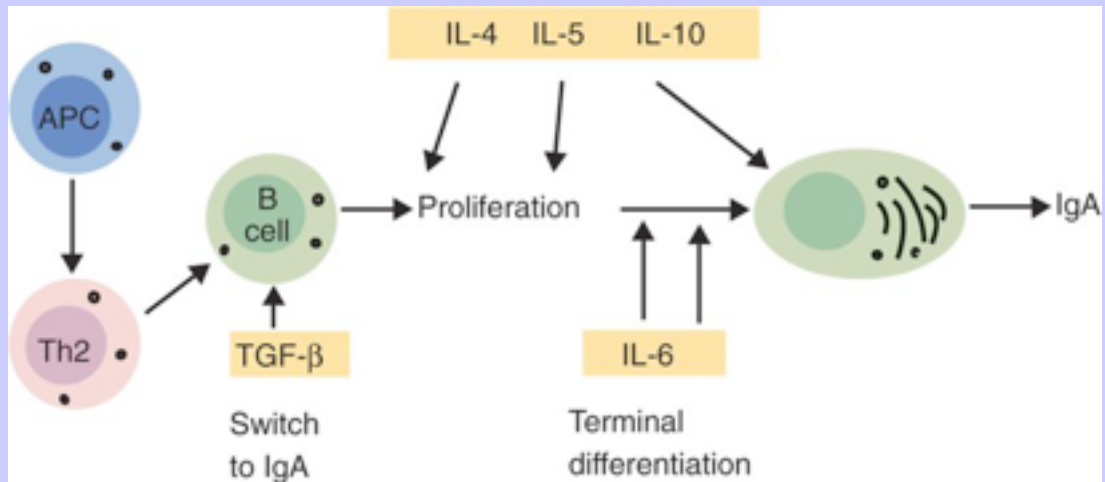


FIGURE 19-11 Immunoglobulin A (*IgA*) is secreted by mucosal plasma cells and binds to receptors (*pIgR*) on the interior surface of intestinal enterocytes. The bound *IgA* is taken into the enterocytes and passed in vesicles to the cell surface. Once in the intestinal lumen, the *pIgR* is cleaved from the cell and remains bound to the *IgA*. In this state it is called secretory component and serves to protect the *IgA* from degradation.

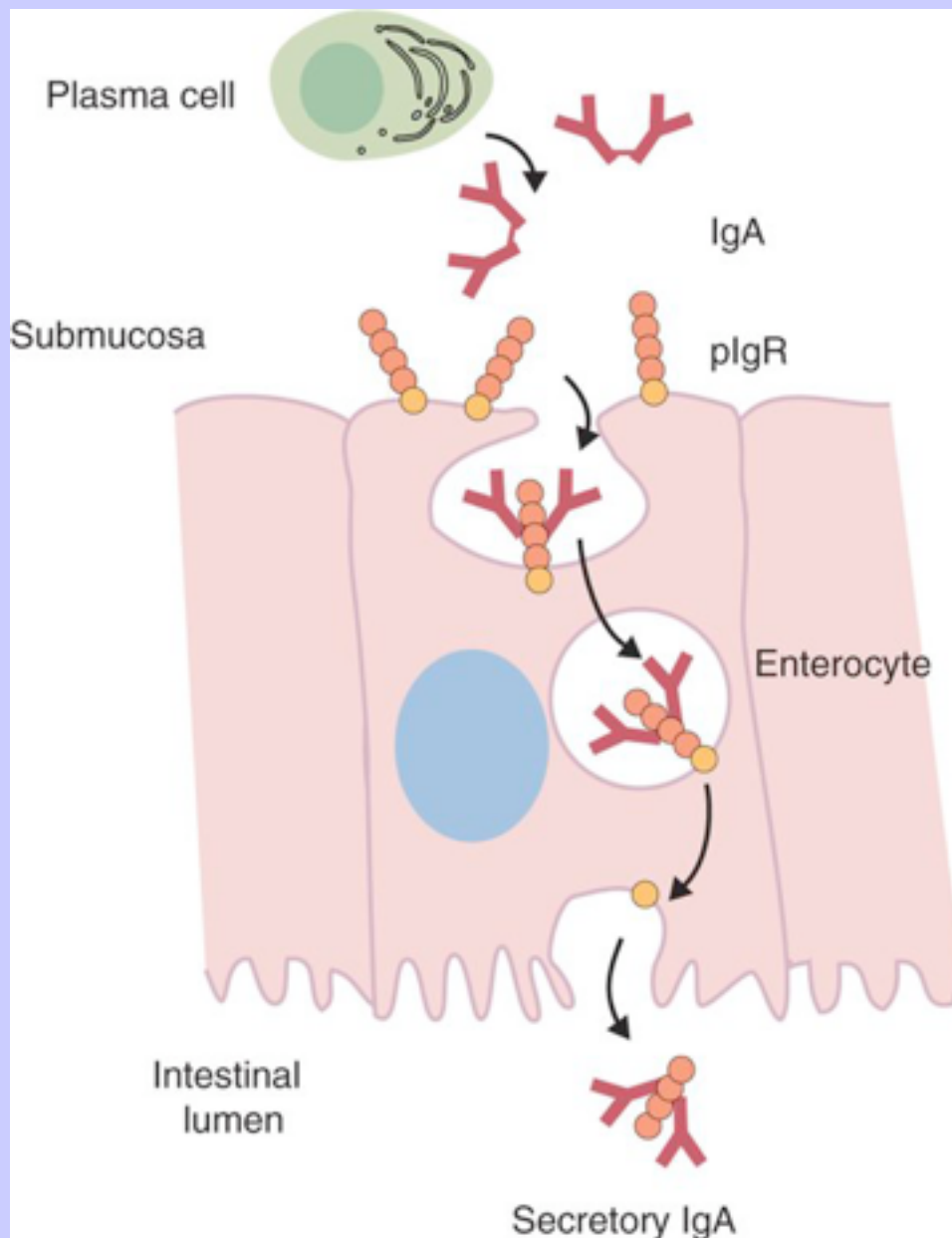


FIGURE 19-12 Immunoglobulin A (*IgA*) is unique in that it can act in three locations. It can bind antigen in tissue fluid or in enterocytes as well as in the intestinal lumen. The bound antigen in tissues or enterocytes is carried to the intestinal lumen.

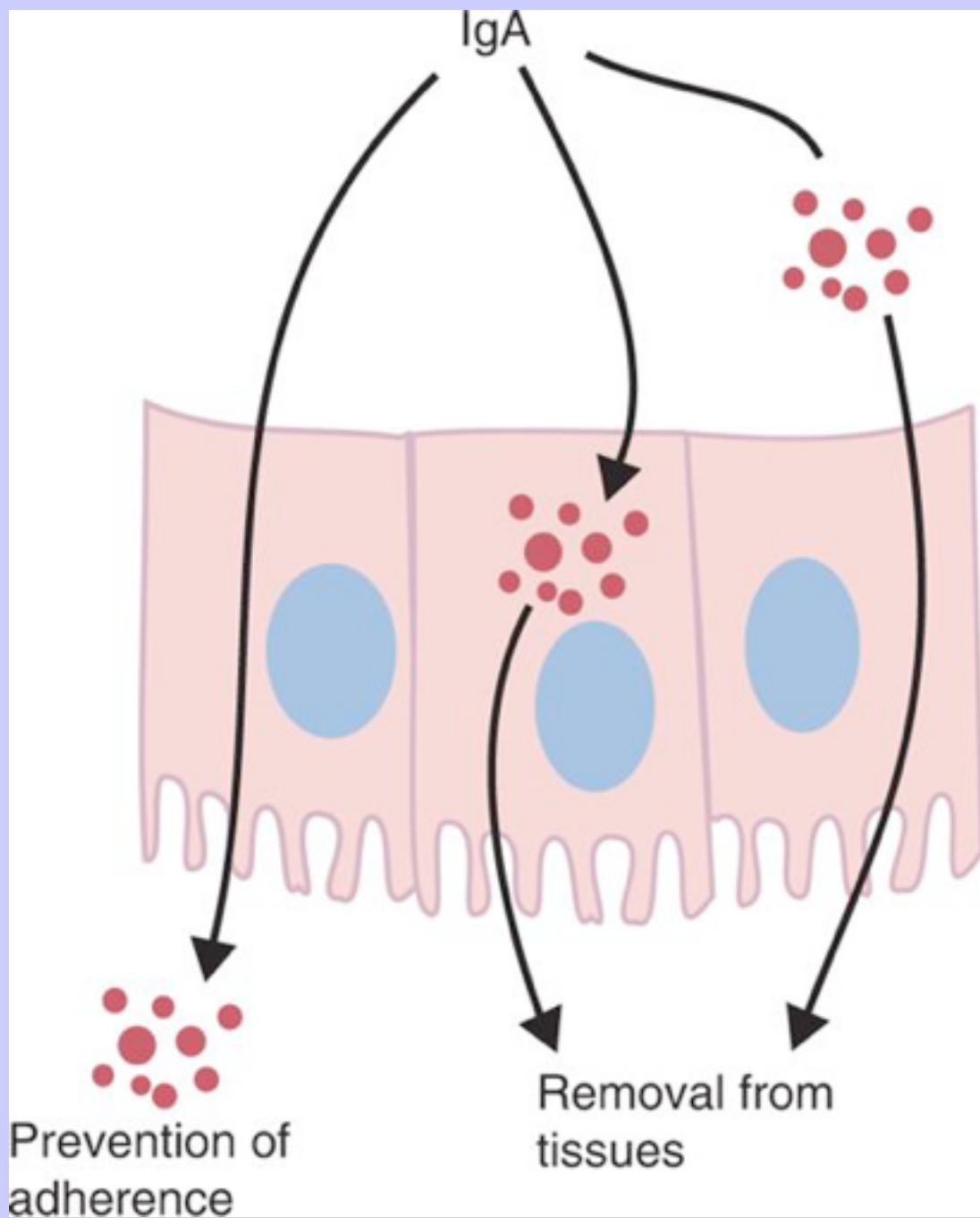
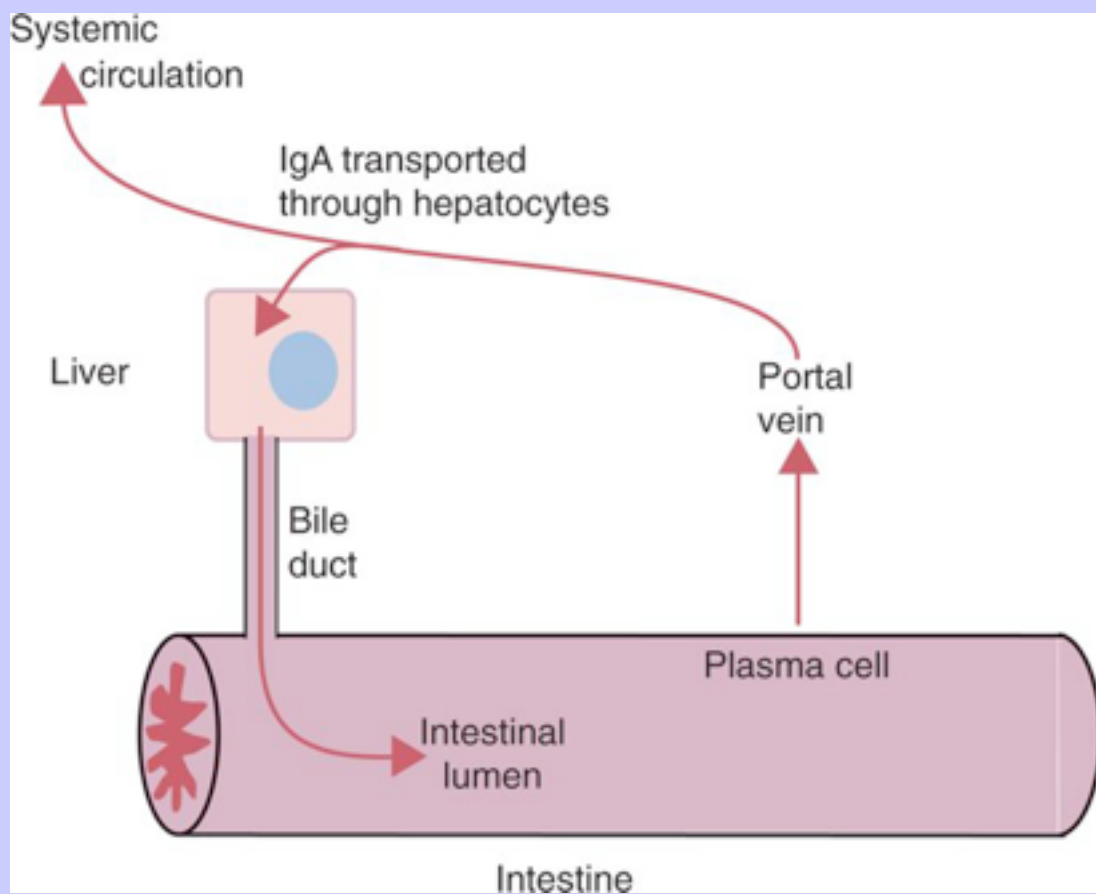


FIGURE 19-13 Some immunoglobulin A (*IgA*), instead of being secreted directly into the intestine, as shown in [Figure 19-11](#), may be carried to the liver, where it is passed through the hepatocytes into the bile duct. In some species, such as the rat, this is a very important pathway. In others it is much less significant. For example, only about 5% of *IgA* reaches the intestine by this route in humans.



systems. Its most important function, however, is immune exclusion, which it achieves by preventing the adherence of bacteria and viruses to epithelial surfaces. If bacteria or viruses cannot adhere to enterocytes, they simply pass along with the intestinal contents and are expelled without doing any harm.

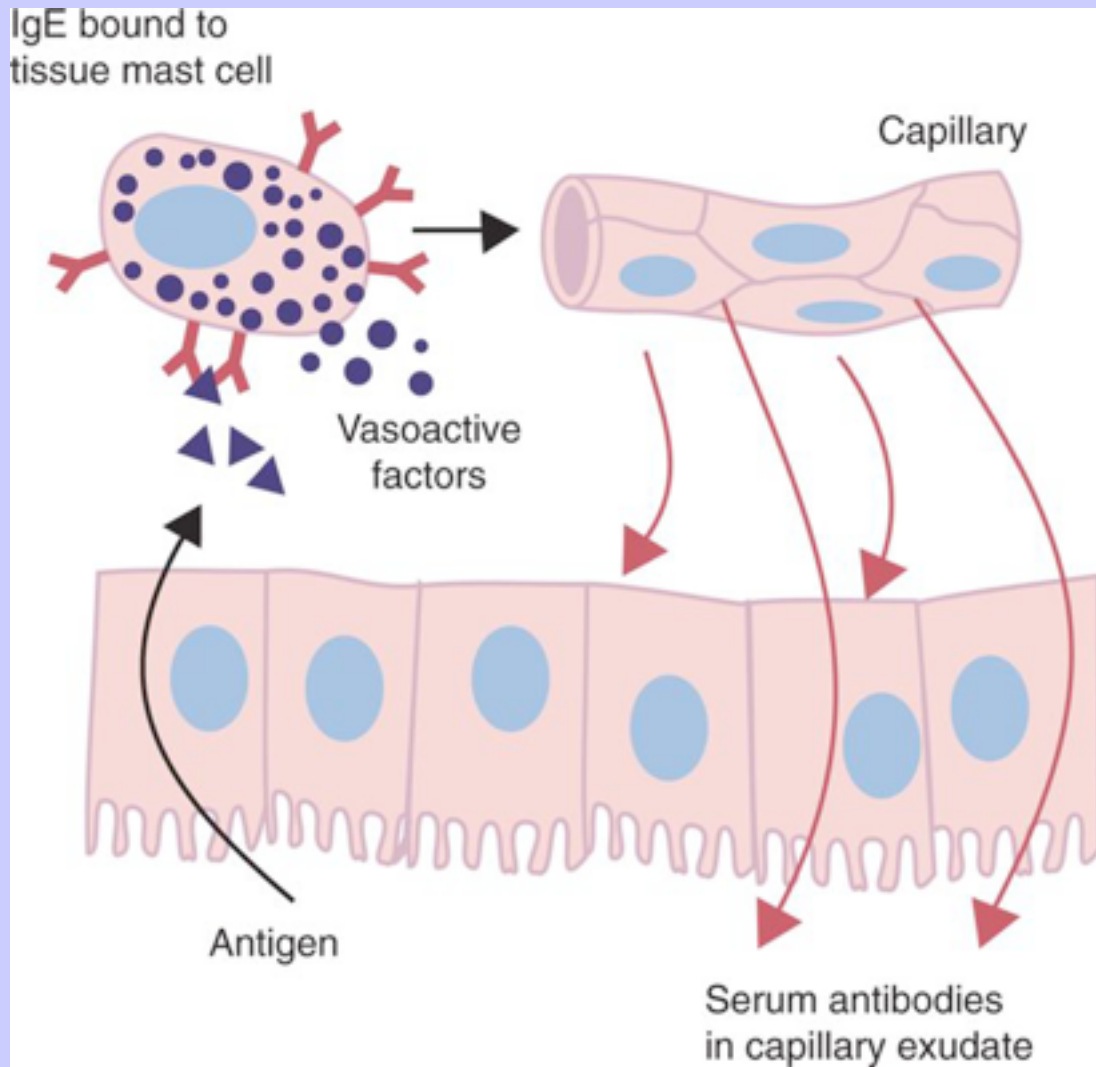
Table 19-1 Approximate IgA Levels in the Serum and Various Secretions of the Domestic Animals

Animal	Secretion (mg/dl)					
	Serum	Colostrum	Milk	Nasal Mucus	Saliva	Tears
Horse	170	1000	130	160	140	150
Cow	30	400	10	200	56	260
Sheep	30	400	10	50	90	160
Pig	200	1000	500	—	—	—
Dog	100	250	400	—	—	—
Cat	200	100	24	—	54	—
Chicken	50	—	—	—	20	15

Because IgA is transported through enterocytes, it can also act inside these cells ([Figure 19-12](#)). Thus IgA can bind to newly synthesized viral proteins inside epithelial cells and interrupt viral replication. In this way, the IgA can prevent viral growth before the integrity of the epithelium is damaged. This is a unique example of an antibody acting in an intracellular location. The second unique function of intracellular IgA is to excrete foreign antigens. Thus IgA can bind to antigens that have penetrated to the submucosa. Once bound, the IgA-antigen complexes will bind to pIgR and be actively transported across the enterocytes into the intestinal lumen. IgA can therefore act at three different levels to exclude foreign antigens: within the submucosa, within enterocytes, and within the intestinal lumen.

In some species, such as rats, rabbits, and chickens, up to 75% of the IgA produced within the intestinal wall may diffuse into the portal blood circulation and be carried to the liver ([Figure 19-13](#)). In these species, hepatocytes express pIgR. The blood-borne IgA thus binds to hepatocytes and is carried across the hepatocyte cytoplasm to be released into the bile canaliculi. Bile is therefore the major route by which IgA reaches the intestine in these species. It is also a route by which antigens bound to circulating IgA can be removed from the body. The reader should note, however, that in the major domestic mammals (dogs, ruminants, and swine), less than 5% of IgA enters the bile.

FIGURE 19-14 The immunoglobulin E (*IgE*) response in the intestinal wall. Antigen reaches *IgE*-sensitized mast cells to cause their degranulation. As a result of this, vasoactive factors are released. These cause increased vascular permeability and exudation of serum *IgG* antibodies.



IgA binds to the pIgR on enterocytes and hepatocytes before being transported to the intestinal lumen or bile. In addition, IgA-antigen complexes can bind to monocytes and macrophages, neutrophils, and eosinophils through the low-affinity receptor FcαR1 (CD89). When IgA-opsonized particles bind to the FcαR on phagocytic cells, they can trigger superoxide production, opsonization, ADCC, and the release of inflammatory mediators. IgA also adheres selectively to Peyer's patch M cells, which appear to possess an IgA receptor that is not FcαR1. This receptor transports IgA from the lumen to the underlying lymphoid tissues and

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explains how an animal may be able to mount a secondary immune response to an antigen in the intestine, even in the presence of IgA.

19.4.1.2

Immunoglobulin M

The earliest immunoglobulins found in the intestine of the newborn are of the IgM class. IgM will also bind to pIgR and is carried through the enterocyte to the lumen. Because of its structure, however, SIgM is much more susceptible than SIgA to proteases.

19.4.2

Immune Elimination

19.4.2.1

Immunoglobulin E

Because IgA does not activate complement, it functions by immune exclusion. There is a second line of defense, however, which destroys antigen that penetrates the mucosal barrier (immune elimination). This process is mediated by IgE. Cells producing IgE are mainly found on body surfaces rather than in the lymph nodes or spleen. IgE attaches to mast cells within the walls of the intestine, respiratory tract, and in the skin. Thus, if invading organisms evade the IgA and gain access to the tissues, IgE-mediated responses will be triggered ([Figure 19-14](#)). These responses involve rapid degranulation of mast cells and the release of their vasoactive molecules into the surrounding tissues. As described in [Chapter 2](#), these vasoactive molecules cause acute inflammation, increase the permeability of small blood vessels, and promote fluid leakage between enterocytes, leading to the outflow of fluid containing large quantities of IgG.

This process occurs, for example, when parasitic worms invade the intestinal mucosa. IgA has little effect on these invaders, so they have no difficulty in burrowing into the superficial layers of the mucosa. When parasite antigens encounter sensitized mast cells, however, the release of vasoactive molecules together with the intense local inflammation, changes in blood flow, and intestinal motility may be sufficient to force the parasite to disengage—a phenomenon called “self-cure” (see [Chapter 24](#)).

Thus IgA and IgE work in concert. IgA normally is the first line of defense, and IgE serves as a back-up system. If IgA production is defective, the IgE response may be triggered to excess. As a result, low levels of IgA result in increased IgE production and the development of allergic responses to food and inhaled antigens.

19.4.2.2

Immunoglobulin G

In ruminants (especially cattle), IgG1, not IgA, is the major secretory immunoglobulin in colostrum and milk. This is due to selective transfer from the bloodstream into the mammary gland. On other body surfaces in ruminants, however, IgA remains the predominant immunoglobulin, although IgG1 is also present at high concentrations. IgG2 is also transferred into the intestine and saliva in ruminants. IgG may be of greater protective significance in the respiratory tract than in the intestine since it is less likely to be degraded by proteases there.

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19.5 IMMUNITY ON SPECIFIC SURFACES

19.5.1 Immunity in the Gastrointestinal Tract

Saliva is rich in IgA and hence protects the mouth against infections. Small amounts of IgG are secreted into the crevicular groove between the gums and the base of the teeth. As a result it has proved possible to make a vaccine against caries-causing bacteria. Immunization of dogs with these organisms reduces microbial colonization of this area and so prevents plaque formation and periodontitis.

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The gastrointestinal mucous barrier is also critical to the defense of body surfaces. Gastrointestinal mucins are glycoproteins secreted into the intestine, where they form a mucous gel. The mucous gel serves as a physical barrier keeping pathogens and chemicals away from the enterocytes. It also acts as a lubricant, blocks chemical insults, and can trap and then expel pathogens. The mucous layer has an inner and outer component. The inner layer is rich in defensins and lysozyme and contains few bacteria, so limiting the ability of even commensal bacteria to bind to epithelial cells. Many enteric pathogens have receptors for mucins, and some may secrete enzymes that destroy mucins.

19.5.1.1 Immunity to Commensals

Vast numbers of bacteria live within the gastrointestinal tract. These commensal bacteria invade the gastrointestinal tract soon after birth and drive the normal development of the immune system in species such as pigs and rabbits. Germ-free animals fail to fully develop their intestinal lymphoid tissues. The intestinal microflora consists of more than 800 bacterial species and in humans about a hundred trillion (10^{14}) bacterial cells. They include Gram-positive and Gram-negative bacteria and facultative aerobic and anaerobic organisms. Healthy living animals successfully exclude these organisms. Immediately after death, however, they can invade the body and are the prime cause of postmortem decomposition. It therefore makes sense that live animals make a significant effort to ensure that this cannot occur before death.

Commensal bacteria largely reside in the intestinal lumen behind a glycocalyx barrier that keeps them from direct contact with enterocytes. Nevertheless, it has been shown that intestinal dendritic cells insert their processes into the intestinal lumen, where they take up commensal bacteria. These bacteria can persist within the dendritic cells for several days. These dendritic cells loaded with commensal bacteria do not penetrate further than mesenteric lymph nodes. This permits the dendritic cells to induce a local IgA response that serves to prevent mucosal penetration by these commensals. These commensal-loaded dendritic cells are restricted to the mucosal region and thus induce an IgA response that is confined to the mucosa. The commensals are prevented from breaching the mucosal barrier by the ongoing IgA response, while the mesenteric lymph nodes form a barrier that prevents the commensals from reaching the systemic immune system and eliciting damaging inflammation. Evidence suggests that the commensal bacteria can actively suppress inflammatory responses in the gut wall. They most likely do this by inhibiting activation of nuclear factor kappa-B (NF- κ B) by blocking degradation of its inhibitor, I κ B. In humans, an excessive immune response to this microflora is a cause of chronic inflammatory bowel disease (IBD). It is likely that a similar type of local IgA response occurs against food antigens; as a result, there is more immune activity in the intestine than in all other lymphoid tissues combined. It has been estimated, for example, that more than 80% of the body's activated B cells are found in the intestine.

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19.5.1.2

Immunity to Food

While it is now fairly clear how the immune response to commensal bacteria is maintained, it remains unclear how immune responses to food are regulated. Secretory IgA responses are not usually generated against food antigens. Likewise, soluble food proteins are unlikely to trigger intense TLR responses. (Although TLR4-deficient mice readily develop food allergies.) It may be that T_{reg} cells play a major role in tolerance to food antigens.

It has been estimated that about 2% of ingested food protein is absorbed as peptide fragments large enough to be recognized by the immune system, although a very much smaller fraction (<0.002%) is absorbed intact. This protein readily reaches the portal circulation, but little passes the liver and enters the systemic circulation. Presumably the Kupffer cells of the liver effectively capture food antigens. Antibodies produced locally may bind to adsorbed antigen and generate immune complexes that are removed as the blood passes through the liver. Thus if a calf is fed a defined dietary antigen such as soy protein, although it is initially well absorbed, the animal soon begins to make IgA antibodies against it. Once these antibodies are produced and immune exclusion develops, the amount of protein absorbed drops significantly. If a new protein is introduced into the feed, it too will be initially absorbed until IgA is produced against it. Thus a prime role for IgA may be to exclude food antigens from the circulation.

The extent to which normal animals make anti-bodies against proteins in their food has been unclear. In one study, cats were fed soy and casein either as unprocessed aqueous suspensions or as a component of canned diets. It was found that the cats produced high levels of IgG and IgA antibodies in their serum against both proteins. In contrast, they did not make detectable salivary IgA responses to unprocessed antigens but did so against the canned casein. Thus cats may respond strongly against certain antigens in their diets, especially if these antigens are present in canned food.

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If a small amount of dietary antigen gains access to the general circulation in adults, a systemic immune response may be prevented by the activities of regulatory T cells and oral tolerance may develop. Oral tolerance may involve either cellular suppression or clonal anergy and is generally directed against Th1 cells. The factor that determines which mechanism is involved in this tolerance is probably the dose of antigen. Low doses invoke active suppression. High doses provoke clonal anergy. To ensure that immune exclusion continues to operate, the intestinal mucosa is rich in contrasuppressor cells (see [Chapter 17](#)).

19.5.1.3

Immunity to Pathogens

Invasive pathogens such as *E. coli* or *Salmonella enterica* may cross the glycocalyx and either attach to enterocytes or release damaging toxins. They may penetrate the intestinal mucosa and gain access to the lacteals and portal vessels; they are subsequently trapped in the mesenteric lymph nodes and liver. Other organisms may enter the body through surface lymphoid tissues. For instance, the epithelium at the bottom of the tonsillar crypts is very thin ([Figure 19-15](#)). Viruses may enter the body by this route and multiply locally in the tonsil before spreading elsewhere.

Pathogenic microbes that penetrate the intestinal wall stimulate a more intense inflammatory response than do commensals. This may be due to differences in penetration of the mucosa and the development of immune elimination rather than immune exclusion. The inflammation may actually assist pathogen penetration by opening up tissue barriers. Proinflammatory T cells such as Th17 cells (see [Chapter 17](#)) may promote this reaction. Oral vaccination may help for only a short period to protect against those diseases to which an

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animal is susceptible. Giving a diet containing killed *E. coli* to calves and pigs has resulted in a reduced incidence of diarrhea, a better feed conversion, and an improved overall health of the animals. Live oral transmissible gastroenteritis vaccine may also be given to pregnant sows to stimulate an intestinal IgA response and seed antibody-producing cells to the udder. This results in the appearance of specific antibodies in colostrum and thus in the protection of suckling piglets.

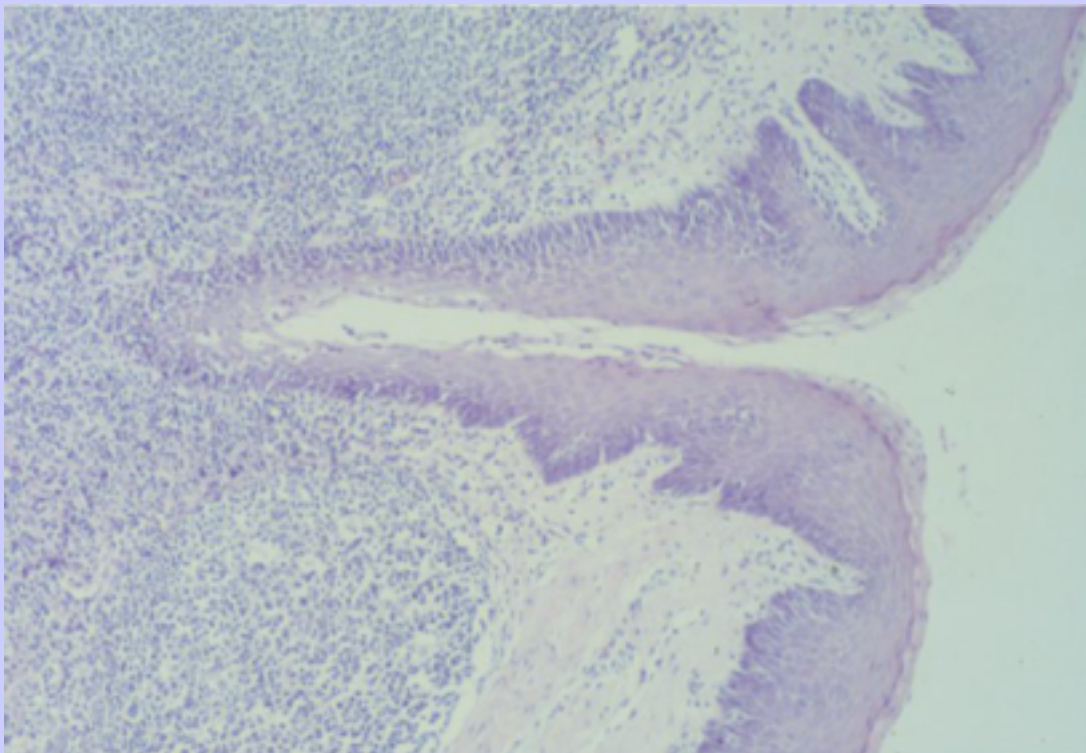
19.5.2

Intestinal Inflammatory Disease

As pointed out above, commensal bacteria growing within the intestine are prevented from invading the gut wall by an ongoing IgA response. They also suppress inflammation by blocking NF- κ B activation. The inhibition of mucosal inflammation is probably also due in part to the presence of IL-10-secreting T_{reg} cells. If these control mechanisms fail, severe inflammation may result and make the intestine much more susceptible to bacteria-induced injury ([Box 19-1](#)).

Canine IBDs are characterized by persistent or recurrent gastrointestinal disease of undetermined cause. The most common form is lymphocytic-plasmacytic enteritis. As its name implies, it is associated with extensive infiltration of the lamina propria of the small intestine by lymphocytes and plasma cells. The disease presents with a history of chronic

FIGURE 19-15 A section of pig tonsil showing a tonsillar crypt. Note how thin the epithelium is at the base of the crypt. An easy invasion route for many organisms. Original magnification $\times 150$. (Courtesy Dr. S. Yamashiro.)



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vomiting, diarrhea, and weight loss. It is associated with an increase in T cell and IgA⁺ plasma cells in the small intestine. The T cells are primarily α/β CD4⁺ cells. There is also an increased number of intestinal mast cells. Affected small intestine shows increased mRNA for IL-12, IFN- γ , tumor necrosis factor- α (TNF- α), and TGF- β . A hypoallergenic diet may result in significant clinical improvement and strongly suggests that the disease results from a food hypersensitivity. It may also respond well to glucocorticoids and the immunosuppressive drug azathioprine. A monoclonal gammopathy has been associated with this condition. Lymphocytic-plasmacytic enteritis has also been described in cats, horses, and a cow.

19.5.2.1

Box 19-1 How to Suppress Intestinal Inflammation

Bacteria that seek to reside peacefully within the intestine must prevent an inflammatory response if they are to survive. *Salmonella enterica pullorum*, the cause of fowl typhoid, is such an organism. It suppresses inflammation by preventing activation of the transcription factor nuclear factor kappa-B (NF- κ B). NF- κ B is normally bound to its inhibitor I κ B so that it cannot move to the nucleus and activate genes. If I κ B is phosphorylated, ubiquitinated, and degraded, NF- κ B is released. The NF- κ B then moves to the nucleus and switches on the genes for various cytokines and chemokines that serve as inflammatory mediators. *S. enterica pullorum* prevents the ubiquitination and degradation of I κ B. As a result NF- κ B remains inhibited, and tissues invaded by *S. enterica pullorum* do not become inflamed. This pathway may well be employed by other members of the normal gut flora to prevent excessive inflammation in the gut wall.

Histiocytic ulcerative colitis in boxers is a very severe form of IBD. The lesions are characterized by the presence of large macrophages that stain intensely with periodic acid–schiff stain. It is possible that this disease is triggered by an unidentified infectious agent since it somewhat resembles Johne's disease. The lesions also show increases in IgG⁺ plasma cells, MHC class II⁺ cells, macrophages, and granulocytes.

Immunoproliferative enteropathy of basenji dogs is an inherited autosomal disease. The disease presents as gastric mucosal hypertrophy, with lymphoid cell infiltration and ulceration. The whole small intestine may show villous blunting, crypt elongation, and infiltration of the mucosa with lymphocytes, plasma cells, and some neutrophils. Dogs show a polyclonal increase in serum IgA. The disease may be controlled by high doses of corticosteroids.

Protein-losing enteropathy of soft coated wheaten terriers is also an inherited disease. Histological examination shows an IBD. The cellular infiltrates are mainly lymphocytes and plasma cells, but neutrophils and eosinophils are often present. This disease may result from food hypersensitivity, possibly to wheat gluten.

Gluten sensitive enteropathy of Irish Setters is a small intestinal disease also caused by exposure to wheat gluten. It is an autosomal recessive condition. As with the diseases described above, affected small intestine is infiltrated with lymphocytes and other inflammatory cells. The mucosa shows increased numbers of CD4⁺ cells and decreased CD8⁺ T cell numbers. Affected dogs may also have elevated serum IgA levels.

19.5.3

Immunity in the Mammary Gland

The protective mechanisms of the udder are presumably not at their most effective in that biological anomaly, the modern dairy cow. The flushing action of the milk helps to prevent invasion by some potential pathogens while milk itself contains bacterial inhibitors (lactenins) and phagocytic cells. In addition, milk contains IgA, secretory component, and IgG1. The IgA and secretory component are closely associated with the milk fat globules. In simple-stomached animals, IgA predominates, whereas in ruminants, IgG1 does.

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IgA is locally synthesized in the mammary tissue, although many of the IgA-producing cells in the gland are derived from precursors originating in the intestine. These cells are a source of antibodies against intestinal pathogens. IgG1, in contrast, is selectively transferred by active transport from serum using the FcRn receptor on mammary gland epithelial cells.

If antigen is infused into a lactating mammary gland, it tends to be promptly flushed out again in the milk. If it is infused into a nonlactating gland, then a local immune response develops in which IgA and IgG1 predominate. Unfortunately, because of the continuous removal of milk, antibody concentrations in this fluid remain low (<100 mg/dl) even though, over a period of time, the amount of immunoglobulin produced by the udder may be considerable. In acute mastitis, the inflammatory response leads to the influx of actively phagocytic cells, especially neutrophils, and to the exudation of serum proteins. As a result immunoglobulin levels in mastitic milk may rise to levels at which they can exert a protective influence (≈ 8000 mg/dl).

Because the local immune response in the udder is relatively ineffective in preventing infection, attempts to vaccinate against mastitis-causing organisms have been generally unsuccessful. Nevertheless, recent advances have produced encouraging results. Thus a *Staphylococcus aureus* vaccine that stimulates the production of antibodies against the pseudocapsule appears to be effective. This pseudocapsule interferes with the ability of milk leukocytes to phagocytose *S. aureus*. Antibodies induced by the vaccine promote opsonization and destruction of the bacteria. A vaccine designed to stimulate antibody production against staphylococcal toxin, as well as the pseudocapsule, has reduced the incidence of mastitis following a challenge infection by 50%. Encouraging results have also been obtained by the use of a J5 mutant vaccine against coliform bacteria (see [Chapter 22](#)). The vaccine, given to cattle at drying off, 30 days later, and at calving, appears to be highly effective.

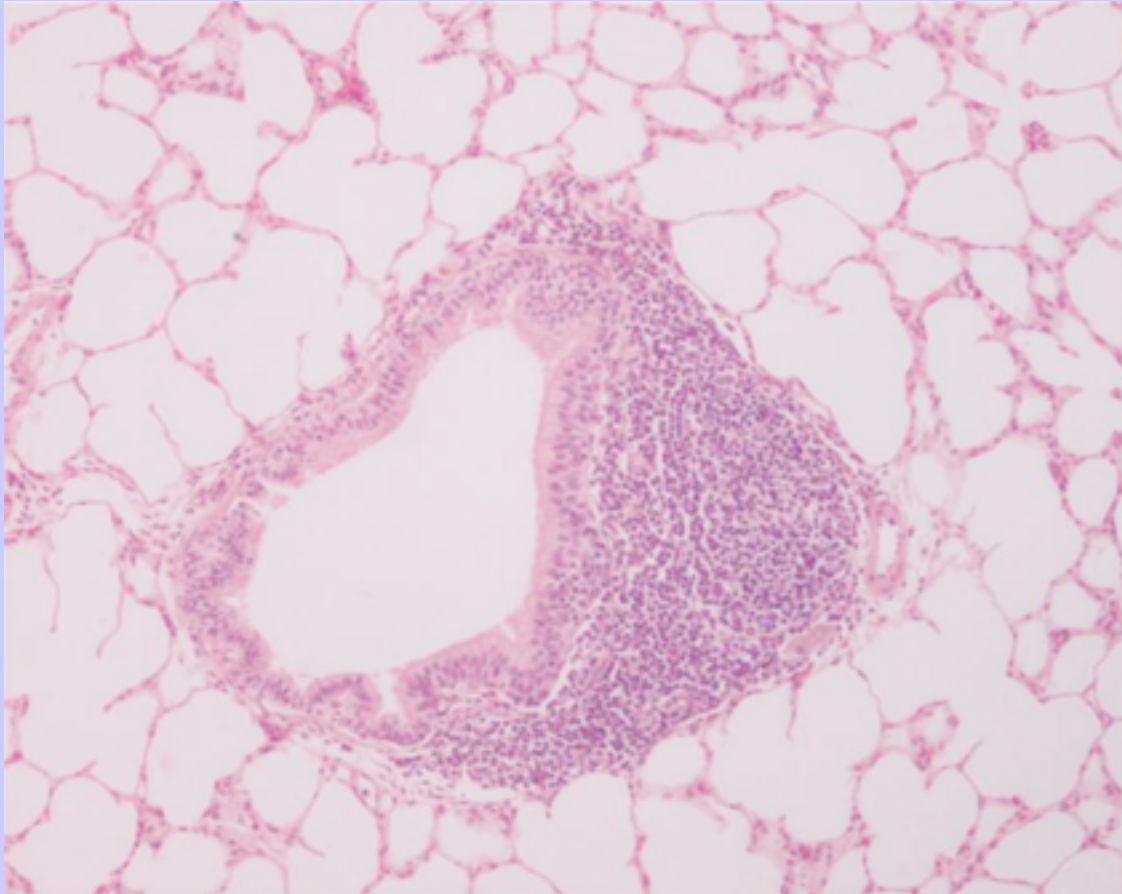
Colostrum is rich in macrophages and lymphocytes. These macrophages can process antigen, and when cultured, their supernatant fluids can enhance IgA production from blood lymphocytes. Milk lymphocytes may survive for some time in the intestine and may transfer some immunity to the newborn animal (see [Chapter 18](#)).

19.5.4 Immunity in the Urogenital Tract

The predominant immunoglobulin in cervico-vaginal mucus is IgA, whereas within the uterus it is IgG. If

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FIGURE 19-16 Lymphoid follicle found in the bifurcation of an airway in a section of calf lung. This type of bronchus-associated lymphoid tissue is a key component of the defenses of the respiratory tract. (From a specimen kindly provided by Drs. N.H. McArthur and L.C. Abbott.)



bacteria such as *Campylobacter fetus* infect the genital tract, vaginal IgA antibodies immobilize and agglutinate the organisms. If the mucous membrane becomes inflamed, IgG antibodies from serum will also assist in protection. *C. fetus* infections are associated with the presence of many mononuclear cells, as well as with delayed skin reactions (type IV hypersensitivity), and it is possible that cell-mediated immunity is also involved in resistance to this local infection. Similar local immune responses may also be directed against other organisms that cause infections of the cervix and vagina, and the presence of agglutinating antibodies in vaginal mucus may be used as a diagnostic test for brucellosis, campylobacteriosis, and trichomoniasis. (The local immune response to trichomoniasis is largely mediated by IgE; see [Chapter 24](#).) Preputial washings of bulls infected with *C. fetus* may contain agglutinins. These are largely IgG1 with some IgM and IgA. IgA is present in small amounts in normal urine, produced presumably by lymphoid tissues in the walls of the urinary tract. In nephritis cases, however, IgG may also be found in large amounts in urine because of the breakdown in the glomerular barrier. Surfactant protein-A is also important in protecting the vagina from infection.

19.5.5 Immunity in the Respiratory Tract

Unlike the intestine, which is exposed to large quantities of foreign material on a daily basis, the respiratory tract is exposed to very small amounts of foreign material, usually in the form of inhaled dusts or aerosols. Large particles are usually trapped in the upper respiratory tract, and only the smallest actually penetrate to the lung. Indeed, the lungs are normally sterile. The respiratory tract contains lymphoid nodules in the walls of the bronchi, as well as lymphocytes distributed diffusely throughout the lung and the walls of the airways ([Figure 19-16](#)). The mucosa of the larynx contains many immunologically active cells including large numbers of T cells. M cells may be associated with these lymphoid nodules, as well as with nasal mucosal lymphoid tissues. The immunoglobulin synthesized in these tissues is mainly secretory IgA, especially in the upper regions of the respiratory tract. This IgA is bound to the mucous layer through secretory component and so enhances the clearance of adherent bacteria. pIgR is expressed at low levels on bronchial epithelial cells. In the bronchioles and alveoli, however, the secretions contain a large proportion of IgG, the concentration of which is intermediate between the levels in the trachea and in serum. IgE is also synthesized in significant amounts in the lymphoid tissues of the upper respiratory tract. As on other body surfaces, IgA in the respiratory tract probably protects by immune exclusion, whereas IgG and IgE act by immune elimination (see [Figure 19-14](#)).

The mucous layer that lines the airways contains a complex mixture of antimicrobial molecules. These include lysozyme, lactoferrin, surfactant proteins, and cationic peptides such as the defensins and cathelicidins. Most microorganisms that encounter this mucous layer are likely to be killed rapidly.

Pulmonary surfactants are soaplike lipoprotein complexes that reduce lung surface tension and make breathing easier. They are also critical components of the lung defenses, enhancing pathogen clearance and regulating pulmonary immunity. There are four major surfactants: A, B, C, and D. Surfactants B and C are extremely hydrophobic and reduce surface tension at the alveolar surface so that a thin film forms on the surface and prevents lung collapse. Surfactant proteins A and D are collectins—carbohydrate-binding lectins that bind microbial surface carbohydrates. They are potent opsonins for most pulmonary bacteria as well as for some viruses. These surfactants facilitate phagocytosis, activate macrophages, promote chemotaxis, enhance the respiratory burst, and promote the production of inflammatory cytokines. They also regulate the production of inflammatory mediators by immune cells. SP-A thus regulates the production of TNF- α , the respiratory burst, and NO. Surfactant proteins enhance the clearance of apoptotic cells from the lung. This is especially important in the resolution of inflammation, where apoptotic neutrophils should be removed by macrophages as promptly as possible. Surfactant proteins can also modulate the functions of dendritic cells and T cells. SP-A inhibits the maturation of dendritic cells while SP-D enhances their uptake and presentation of antigen. Both SP-A and SP-D inhibit T cell proliferation. In pigs suffering from bronchopneumonia, affected tissues produce increased amounts of SP-D. This SP-D is expressed in the alveolar surfactant layer and localized on the surface of alveolar macrophages as well as within the cytoplasm of dendritic cells.

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Table 19-2 Composition of Cells in Canine Bronchoalveolar Lavage Fluid

Cell	Percentage (Range)
Macrophages	79.4 (71-87)
Lymphocytes	13.5 (7-20)
Eosinophils	3.6 (0-14)
Mast cells	2.1 (0-5)
Epithelial cells	0.8 (0-6)
Neutrophils	0.6 (0-2)
Lymphocyte Percentages	
T cells	52.0 (34-69)
CD4 ⁺	21.9 (10-32)
CD8 ⁺	17.8 (6-25)
CD4/CD8 ratio	1.3 (0.8-2.4)
From Vail DM, Mahler PA, Soergel SA: <i>Am J Vet Res</i> 56:282-285, 1995.	

Many cells may be washed out of the airways of the lung by lavage with saline. In dogs, about 80% of bronchoalveolar cells are macrophages and 13% are lymphocytes, of which about half are T cells ([Table 19-2](#)). In healthy horses, about 50% of the cells in bronchoalveolar washes are macrophages, 40% are lymphocytes, and 2% are neutrophils. In sheep, B cells are less than 10% of the lung lymphocyte population. Lung T cells can produce cytokines, and alveolar macrophages are activated following infection with *Listeria monocytogenes*. Cell-mediated immune reactions are therefore readily provoked among the cells within the lower respiratory tract.

The lungs of the majority of domestic species (pigs, horses, sheep, goats, cattle, cats) differ from rodent, human, or dog lungs in that they contain large numbers of intravascular macrophages (see [Chapter 4](#)). It has been estimated that these macrophages cover 16% of the lung capillary surface in young pigs. As a result of the presence of these cells, the lungs of these species can clear more bacteria from the blood than can the liver and spleen. In pigs, pulmonary intravascular macrophages are damaged by porcine reproductive and respiratory syndrome virus. As a result, infected animals are more likely to develop *S. suis* pneumonia. There is debate as to whether lung macrophages are effective antigen-presenting cells. A dense network of dendritic cells is found within airway epithelium and alveoli.

19.5.6 Immunity on the Skin

The skin is the first line of defense against many microbial invaders. It carries out this function effectively; few bacteria can penetrate intact skin unaided. The skin has a multitude of innate defenses ranging from its own microbial flora to the production of potent antimicrobial peptides. It also participates in the acquired immune system through the use of the Langerhans cell network. These Langerhans cells can bind exogenous antigen and may present it to nearby helper T cells. In the domestic species most of the epidermal T cells have γ/δ TCRs. In cattle, for example, 44% of dermal T cells are γ/δ -positive.

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A T cell subpopulation selectively homes to the skin when attracted by sunlight-induced vitamin D. If an antigen is injected intradermally, such as occurs when a tick bites an animal, the antigen is trapped by Langerhans cells and presented to these skin T cells, thus stimulating a rapid and effective immune response. A similar reaction occurs when reactive chemicals are painted on the skin. If skin is subjected to severe ultraviolet irradiation, the Langerhans cells may be destroyed and the protective mechanisms in the skin are effectively suppressed. Skin washings contain immunoglobulins. For example, in cattle, serum IgM, IgG1, and IgG2 cross the skin by transudation, but the IgA appears to be locally synthesized.

19.6 VACCINATION ON BODY SURFACES

When animals are vaccinated against organisms that cause local infections of body surfaces, such as the intestinal or respiratory tracts, it makes sense to stimulate an IgA response. To do this, the vaccine antigen must be applied locally. Unfortunately, this is not always easy. Inactivated antigens are usually ineffective in triggering an IgA response since they are immediately washed or sneezed off when applied to mucous membranes. (A notable exception occurs when high levels of vaccine antigens are incorporated in feed.) The only way a significant IgA response can be triggered is to use live vaccines, where vaccine organism can temporarily invade mucous membranes. The vaccine must persist for a sufficient time to trigger an immune response yet not cause significant damage. Good examples of such vaccines are the respiratory tract vaccines against bovine or feline rhinotracheitis. Even some of these vaccines may cause a transient conjunctivitis or tracheitis. Other examples of effective live oral vaccines include polio vaccine in humans and transmissible gastroenteritis vaccine in piglets.

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Systemic vaccination against these surface infections will provide limited immunity, since small quantities of IgG may be transferred from serum to the mucosal surface. Indeed, many currently available vaccines simply work by stimulating high levels of IgG antibodies in blood. These are effective, because once an invading organism causes tissue damage and triggers an inflammatory response, the site of invasion is flooded by IgG. Nevertheless, this is clearly not an efficient way of providing immunity.

Once a protective IgA response has been stimulated, other difficulties may arise. For example, secondary immune responses are sometimes difficult to induce on surfaces, and multiple doses of vaccine may not increase the intensity or duration of the local immune response. This is not caused by any intrinsic defect but occurs because high levels of IgA can block antigen absorption and prevent it from reaching antigen-presenting cells.

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²⁰ CHAPTER 20 Vaccines and Their Production

^{20.1} KEY POINTS

- Passive immunization using preformed antibodies made in a normal animal provides immediate protection but the resulting immunity is short-lived.
- Active immunization using live or dead vaccines produces slowly developing but long-lasting immunity.
- Live vaccines tend, in general, to stimulate a more effective immune response than vaccines containing killed organisms. However, vaccines containing killed organisms tend to be somewhat safer.
- Innovative molecular techniques such as the use of DNA vaccines may permit the development of vaccines against diseases where current vaccines are ineffective.
- Adjuvants are substances added to vaccines to enhance their effectiveness.

Vaccination is by far the most efficient and cost-effective method of controlling infectious diseases in humans and animals. The eradication of smallpox from the globe, the elimination of hog cholera and brucellosis from many countries, as well as the control of diseases such as foot-and-mouth disease, canine distemper, pseudorabies, and rinderpest would not have been possible without the use of effective vaccines. Vaccine technology continues to advance rapidly, especially through the use of modern molecular techniques and with our increased understanding of immune mechanisms and ways to optimize immune responses to achieve maximal protection.

^{20.2} TYPES OF IMMUNIZATION PROCEDURES

There are two basic methods by which any animal may be made immune to an infectious disease ([Figure 20-1](#)): passive and active immunization. Passive immunization produces temporary immunity by transferring antibodies from a resistant to a susceptible animal. These passively transferred antibodies give immediate protection, but since they are gradually catabolized, this protection wanes and the recipient eventually becomes susceptible again.

Active immunization, in contrast, involves administering antigen to an animal so that it responds by mounting an immune response. Reimmunization or exposure to infection in the same animal will result in a secondary immune response and greatly enhanced immunity. The disadvantage of active immunization is that, as with all acquired immune responses, protection is not conferred immediately. However, once established, immunity is long-lasting and capable of restimulation ([Figure 20-2](#)).

FIGURE 20-1 A classification of the different types of acquired immunity and of the methods employed to induce protection.

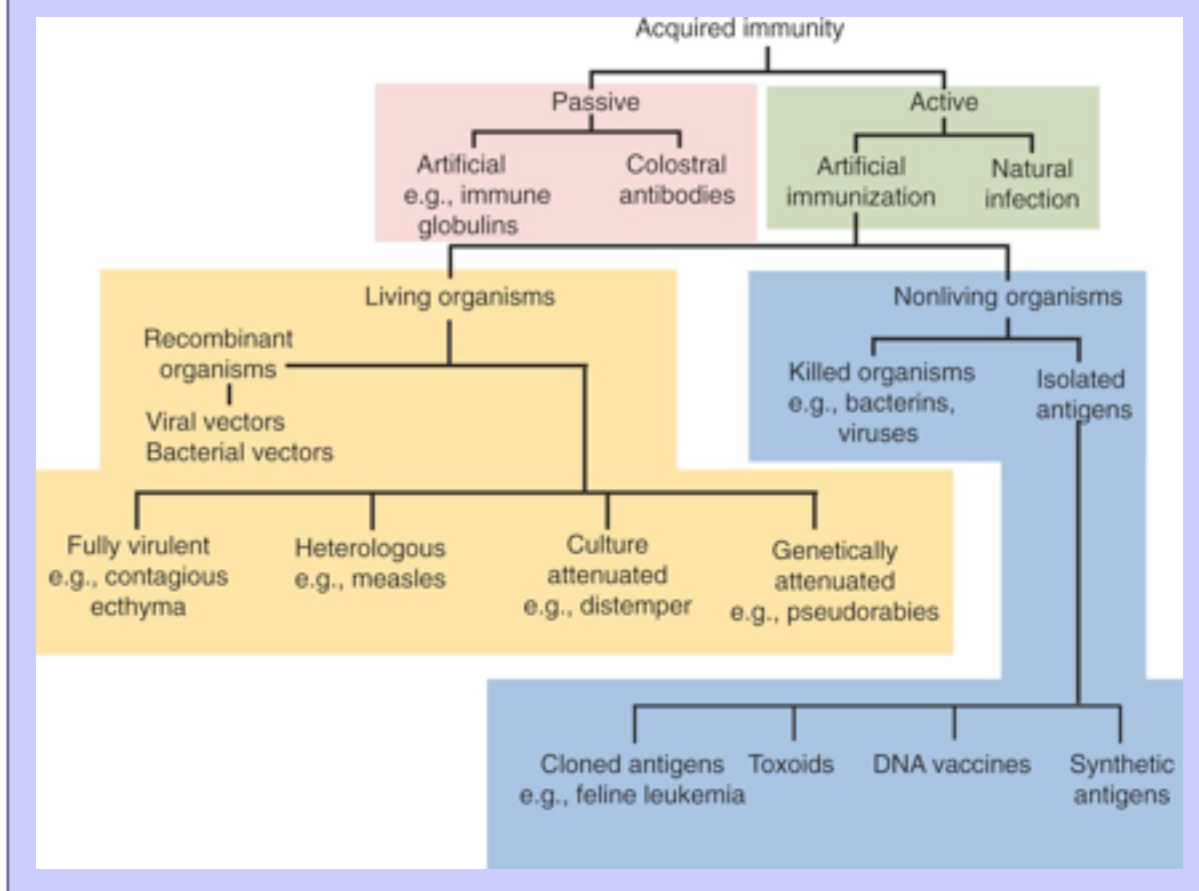
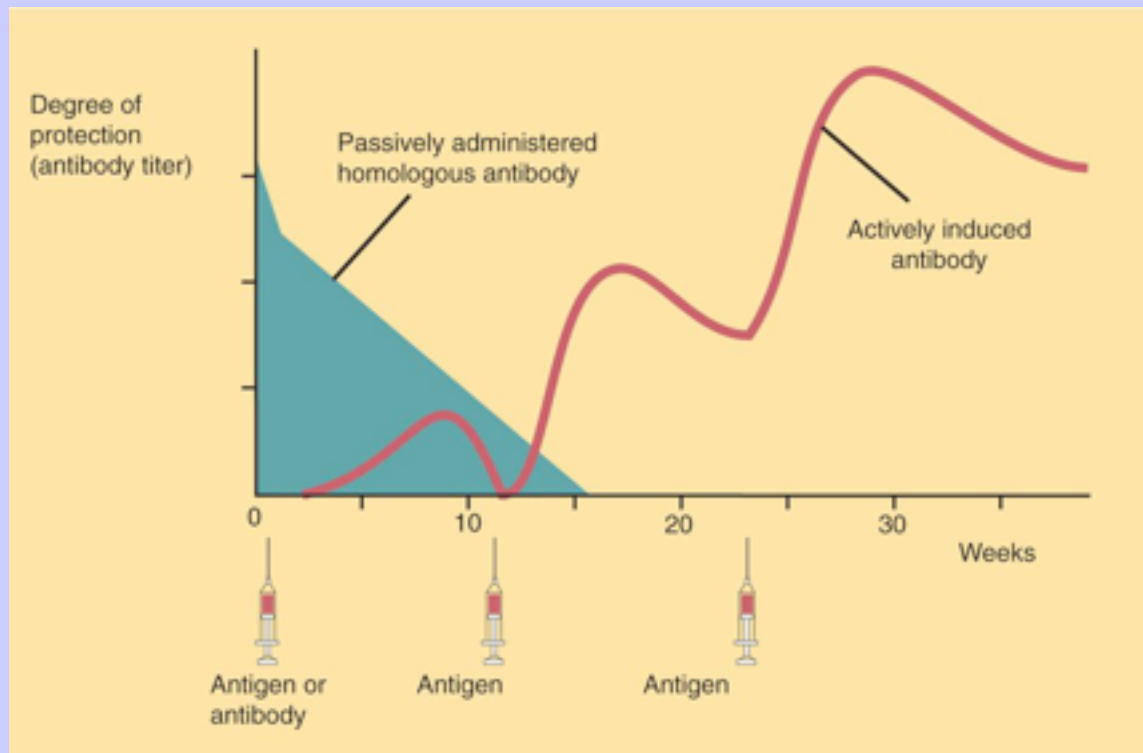


FIGURE 20-2 The levels of serum antibody (and hence the degree of protection) conferred by active and passive methods of immunization.

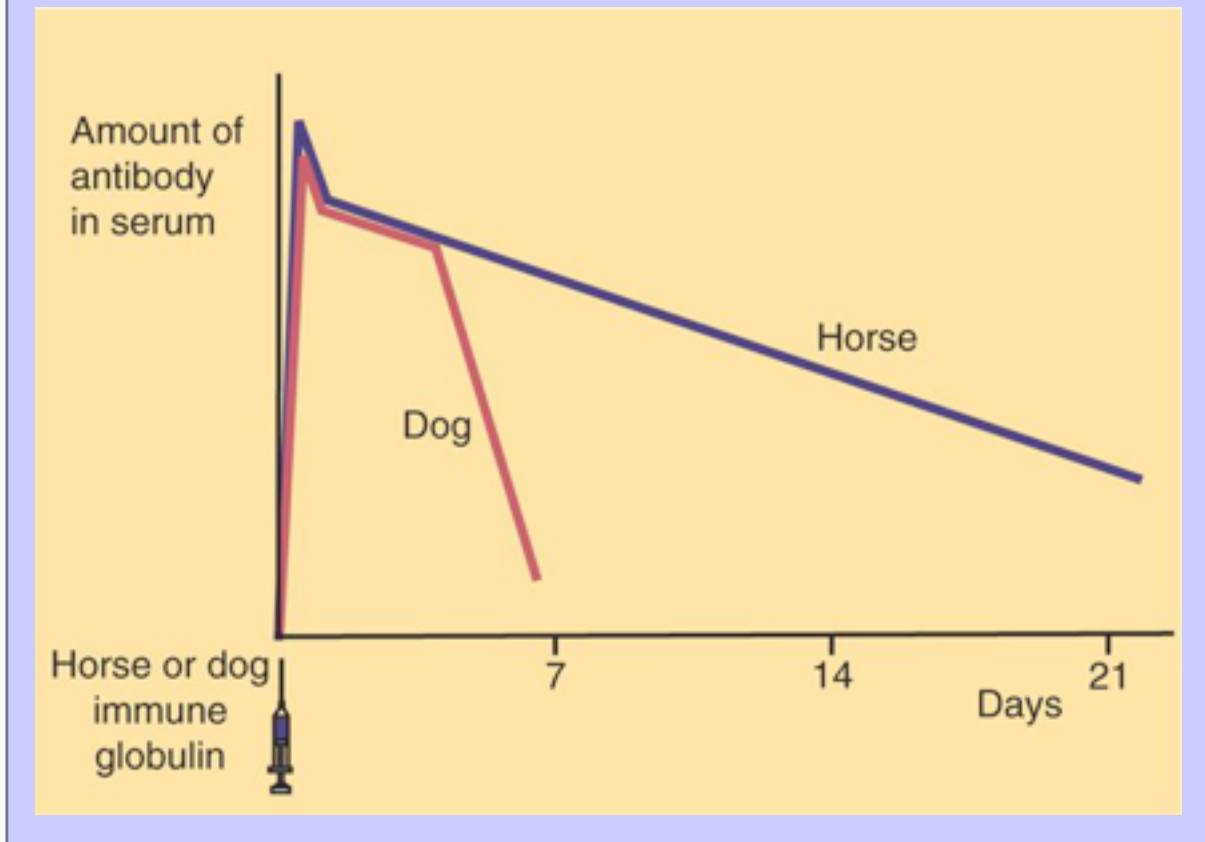


20.3 PASSIVE IMMUNIZATION

Passive immunization requires that antibodies be produced in a donor animal by active immunization and that these antibodies be given to susceptible animals to confer immediate protection. Serum containing these antibodies (antisera) may be produced against a wide variety of pathogens. For instance, they can be produced in cattle against anthrax, in dogs against distemper, in cats against panleukopenia, and in humans against measles. They are most effective protecting animals against toxigenic organisms such as

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FIGURE 20-3 The fate of passively administered immune globulin when given to a homologous species (horse) or to a heterologous species (dog).



Clostridium tetani or *Clostridium perfringens*, using antisera raised in horses. Antisera made in this way are called immune globulins and are commonly produced in young horses by a series of immunizing injections. The toxins of the clostridia are proteins that can be denatured and so made nontoxic by treatment with formaldehyde.

Formaldehyde-treated toxins are called toxoids. Donor horses are first injected with toxoids, but once antibodies are produced, subsequent injections may contain purified toxin. The responses of the horses are monitored, and once their antibody levels are sufficiently high, they are bled. Bleeding is undertaken at intervals until the antibody level drops, when the animals are again boosted with antigen. Plasma is separated from the horse blood, and the globulin fraction that contains the antibodies is concentrated, titrated, and dispensed.

20.3.1 Box 20-1 Serum Hepatitis of Horses

On rare occasions, horses may develop acute hepatic necrosis 30 to 70 days after vaccination. It has followed administration of horse plasma, equine immune globulin against tetanus, anthrax, strangles, influenza, and equine encephalitis. It has also occurred following active immunization against equine encephalitis and rhinopneumonitis, where the vaccines were prepared using fetal equine cells. Certain serum mixtures or a single vaccine batch may be associated with a high incidence of the disease. Its etiology, mechanism of transmission, and pathogenesis are unknown. Occasional cases have been described in untreated horses living with affected animals, suggesting that a virus may transmit the disease. Nevertheless, experimental transmission and serological testing have failed to reveal a causal agent. The disease is severe, with 53% to 88% mortality.

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Clinical signs include anorexia, icterus, excessive sweating, and neurological abnormalities. Clinical chemistry confirms severe liver damage with high liver enzyme levels, ammonia, and bilirubin.

To standardize the potency of different immune globulins, comparison must be made with an international biological standard. In the case of tetanus immune globulin, this is done by comparing the dose necessary to protect guinea pigs against a fixed amount of tetanus toxin with the dose of the standard preparation of immune globulin required to do the same. The international standard immune globulin for tetanus toxin is a quantity held at the State Serum Institute in Copenhagen. An international unit (IU) of tetanus immune globulin is the specific neutralizing activity contained in 0.03384 mg of the international standard. The U.S. standard unit (AU) is twice the international unit.

Tetanus immune globulin is given to animals to confer immediate protection against tetanus. At least 1500 IU of immune globulin should be given to horses and cattle; at least 500 IU to calves, sheep, goats, and swine; and at least 250 IU to dogs. The exact amount should vary with the amount of tissue damage, the degree of wound contamination, and the time elapsed since injury. Tetanus immune globulin is of little use once the toxin has bound to its target receptor and clinical signs appear.

Although immune globulins give immediate protection, some problems are associated with their use. For instance, when horse tetanus immune globulin is given to horses, it will persist for a relatively long time, being removed only by catabolism. If, however, it is given to a cow or dog, the horse proteins will be perceived as foreign, elicit an immune response, and be rapidly eliminated ([Figure 20-3](#)). To reduce antigenicity, immune globulins are usually treated with pepsin to destroy their Fc region and leave intact only the portion of the immunoglobulin molecule required for toxin neutralization—the F(ab)₂ fragment.

If circulating horse antibody is still present by the time the recipient animal mounts an immune response, the immune complexes formed may cause a type III hypersensitivity reaction called serum sickness (see [Chapter 27](#)). If repeated doses of horse immune globulin are given to an animal of another species, this may provoke immunoglobulin E (IgE) production and anaphylaxis (see [Chapter 25](#)). Finally, the presence of high levels of circulating horse antibody may interfere with active immunization against the same antigen. This is a phenomenon similar to that seen in newborn animals passively protected by maternal antibodies ([Box 20-1](#)).

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Monoclonal antibodies are another source of passive protection for animals. These are, however, mainly made by mouse-mouse hybridomas and thus are mouse immunoglobulins. They will therefore stimulate an immune response when given to animals of other species. Nevertheless, mouse monoclonal antibodies against the K99 pilus antigens of *Escherichia coli* can be given orally to calves to protect them against diarrhea caused by this organism. A mouse monoclonal antibody to lymphoma cells has been successfully used in the treatment of dogs with this tumor. Now that methods have been developed to produce monoclonal antibodies from domestic animal cells (xenohybridomas; see [Chapter 13](#)), it is likely that they too will have a place in infectious disease control.

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20.4 ACTIVE IMMUNIZATION

Active immunization has several advantages compared with passive immunization. These include the prolonged period of protection and the recall and boosting of this protective response by repeated injections of antigen or by exposure to infection. An ideal vaccine for active immunization should therefore give prolonged strong immunity. This immunity should be conferred on both the animal immunized and any fetus carried by it. To confer this strong immunity, the vaccine should be free of adverse side effects. The ideal vaccine should be cheap, stable, and adaptable to mass vaccination; ideally, it should stimulate an immune response distinguishable from that resulting from natural infection so that immunization and eradication may proceed simultaneously.

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In addition to the requirements listed above, effective vaccines must have other critical properties. First, antigen must be delivered efficiently so that antigen-presenting cells can process antigen and release appropriate cytokines. Second, both T and B cells must be stimulated so that they generate large numbers of memory cells. Third, helper and effector T cells must be generated to several epitopes in the vaccine so that individual variations in major histocompatibility complex (MHC) class II polymorphism and epitope properties are minimized. Finally, the antigen must be able to stimulate memory cells in such a way that protection will last as long as possible.

20.4.1 Living and Killed Vaccines

Unfortunately, two of the prerequisites of an ideal vaccine—high antigenicity and absence of adverse side effects—are often incompatible. Modified live vaccines infect host cells and undergo viral replication. The infected cells then process endogenous antigen. In this way live viruses trigger a response dominated by CD8⁺ cytotoxic T cells, a Th1 response. This may be hazardous because the vaccine viruses may themselves cause disease or persistent infection (called residual virulence). Killed organisms, in contrast, act as exogenous antigens. They commonly stimulate responses dominated by CD4⁺ Th2 cells. This may not be the most appropriate response to some organisms, but it may be safer. It also appears that dendritic cells respond in a somewhat different fashion to live and killed bacteria. Live organisms (*Salmonella*) induce greater upregulation of CD40, CD86, interleukin-6 (IL-6), IL-12, and granulocyte-macrophage colony-stimulating factor than do killed organisms. This suggests that dendritic cells follow different maturation pathways following exposure to live or killed bacteria.

The practical advantages and disadvantages of vaccines containing living or killed organisms are well demonstrated in the vaccines available against *Brucella abortus* in cattle. *B. abortus* is a cause of abortion in cattle, and vaccination has been used historically to control the disease. *Brucella* infections are best controlled by a T cell-mediated immune response, and a vaccine containing a living avirulent strain of *B. abortus* is required for the control of this infection. Older *Brucella* vaccines, especially strain 19, caused a life-long immunity in cows and successfully prevented abortion. Unfortunately, strain 19 vaccine also caused systemic reactions: swelling at the injection site, high fever, anorexia, listlessness, and a drop in milk yield. Strain 19 could cause abortion in pregnant cows, orchitis in bulls, and undulant fever in humans. To eradicate brucellosis, serological tests are used to identify infected animals, and strain 19 caused an antibody response that was difficult to distinguish from a natural infection.

Because of the disadvantages associated with the use of strain 19, considerable efforts were made to find a better alternative. Unfortunately, killed vaccines (strain 45/20) protected cattle for less than 1 year. More recently, a live attenuated strain of *B. abortus* called RB-51 has been used in cattle in the United States. This is a rough mutant that fails to produce the lipopolysaccharide O antigen. As a result, it produces a strong Th1 response, but unlike strain 19 does not induce false-positive results in the standard diagnostic tests such as card agglutination, complement fixation, or tube agglutination. It is therefore possible to distinguish between vaccinated and infected cattle. RB-51 is less pathogenic for cattle than strain 19, and it is not shed in nasal secretions, saliva, or urine. RB-51 will not cause abortion in pregnant cattle. It will, however, cause disease in accidentally exposed humans, and because of its failure to stimulate antibody production, this may be difficult to diagnose.

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20.4.1.1

Box 20-2 The Relative Merits of Living and Dead Vaccines

Living Vaccines	Inactivated Vaccines
<ul style="list-style-type: none">• Fewer doses required• Adjuvants unnecessary• Less chance of hypersensitivity• Induction of interferon• Relatively cheap• Smaller dose needed• Can be given by natural route• Stimulate both humoral and cell-mediated response• Longer lasting protection	<ul style="list-style-type: none">• Stable on storage• Unlikely to cause disease through residual virulence• Do not replicate in recipient• Unlikely to contain live contaminating organisms• Will not spread to other animals• Safe in immunodeficient patients• Easier to store• Lower development costs• No risk of reversion

The advantages of vaccines such as *Brucella* 45/20 that contain killed organisms are that they are safe with respect to residual virulence and are relatively easy to store, since the organisms are already dead (Box 20-2). These advantages of killed vaccines correspond to the disadvantages of live vaccines such as strain 19 or RB-51. That is, some live vaccines may possess residual virulence, not only for the animal for which the vaccine is made but also for other animals. They may revert to a fully virulent type or spread to unvaccinated animals. Thus some strains of porcine reproductive and respiratory syndrome virus vaccine may be transmitted to unvaccinated pigs, causing persistent infection and disease. Live vaccines always run the risk of contamination with unwanted organisms; for instance, outbreaks of reticuloendotheliosis in chickens in Japan and Australia have been traced to contaminated Marek's disease vaccines. A major outbreak of bovine leukosis in Australia resulted from contamination of a batch of babesiosis vaccine containing whole calf blood. Abortion and death have occurred in pregnant bitches that received a parovirus vaccine contaminated with bluetongue virus. Contaminating mycoplasma may also be present in some vaccines. Scrapie has been spread in mycoplasma vaccines. Finally, vaccines containing living attenuated organisms require care in their preparation, storage, and handling to avoid killing the organisms. Thus maintaining the cold chain can account for 20% to 80% of the cost of a vaccine in the tropics.

The disadvantages of killed vaccines parallel the advantages of living vaccines. Thus, the use of adjuvants to increase effective antigenicity can cause severe inflammation or systemic toxicity, whereas multiple doses or high individual doses of antigen increase the risk of producing hypersensitivity reactions, as well as increasing costs. In general, vaccines containing living organisms tend to induce a stronger Th1-dominated immunity than vaccines containing killed organisms. One reason for this is that the living vaccine virus may invade host cells and induce interferon production, thus conferring early protection on susceptible animals. Killed vaccines are more likely to stimulate a Th2 response. For many organisms, especially viruses, a Th1 response may well be more appropriate.

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20.4.2 Inactivation

Organisms killed for use in vaccines must remain as antigenically similar to the living organisms as possible. Therefore crude methods of killing that cause extensive changes in antigen structure as a result of protein denaturation are usually unsatisfactory. If chemicals are used, they must not alter the antigens responsible for stimulating protective immunity. One such chemical is formaldehyde, which cross-links proteins and nucleic acids and confers structural rigidity. Proteins can also be mildly denatured by acetone or alcohol treatment. Alkylating agents that cross-link nucleic acid chains are also suitable for killing organisms, since by leaving the surface proteins of organisms unchanged, they do not interfere with antigenicity. Examples of alkylating agents include ethylene oxide, ethyleneimine, acetyleneimine, and β -propiolactone, all of which have been used in veterinary vaccines. Many successful vaccines containing killed bacteria (bacterins) or inactivated toxins (toxoids) can be made relatively simply by the use of these agents. Some vaccines may contain mixtures of these components. For example, some vaccines against *Mannheimia hemolytica* contain both killed bacteria and inactivated bacterial leukotoxin.

20.4.3 Attenuation

Virulent living organisms cannot normally be used in vaccines. Their virulence must be reduced so that, although still living, they can no longer cause disease. The process of reducing virulence is called attenuation. The level of attenuation is critical to vaccine success. Underattenuation will result in residual virulence and disease; overattenuation will result in an ineffective vaccine. The traditional methods of attenuation were empirical, and there was little understanding of the changes induced by the attenuation process. They usually involved adapting organisms to growth in unusual conditions so that they lost their adaptation to their usual host. For example, the bacillus Calmette-Guérin (BCG) strain of *Mycobacterium bovis* was rendered avirulent by being grown for 13 years on bile-saturated medium. The strain of anthrax currently used in vaccines was rendered avirulent by growth in 50% serum agar under an atmosphere rich in CO₂ so that it lost its ability to form a capsule. *B. abortus* strain 19 vaccine was grown under conditions in which there was a shortage of nutrients. Unfortunately, genetic stability cannot always be guaranteed in these attenuated strains. Back-mutation may occur, and vaccine organisms may redevelop virulence.

A more reliable method of making bacteria avirulent is by genetic manipulation. For example, a modified live vaccine is available that contains streptomycin-dependent *M. hemolytica* and *Pasteurella multocida*. These mutants depend on the presence of streptomycin for growth. When they are administered to an animal, the absence of streptomycin will eventually result in the death of the bacteria, but not before they have stimulated a protective immune response.

Viruses have traditionally been attenuated by growth in cells or species to which they are not naturally adapted. For example, rinderpest virus, which is normally a pathogen of cattle, was first attenuated by growth in rabbits. Eventually, a successful tissue culture–adapted rinderpest vaccine devoid of residual virulence was developed. Similar examples include the adaptation of African horse sickness virus to mice and of canine distemper virus to ferrets. Alternatively, mammalian viruses may be attenuated by growth in eggs. For example, the Flury strain of rabies was attenuated by prolonged passage in eggs and lost its virulence for normal dogs and cats.

The most commonly used method of virus attenuation has been prolonged tissue culture. It is usual to culture cells from the species to be vaccinated to reduce the side effects resulting from the administration of foreign tissues. In these cases virus attenuation is accomplished by culturing the organism in cells to which they are not adapted. For example, virulent canine distemper virus preferentially attacks lymphoid cells. For vaccine

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purposes, therefore, this virus was cultured repeatedly in canine kidney cells, as a result of which its virulence was lost.

Instead of artificially attenuated organisms, some vaccines use antigenically related organisms normally adapted to another species. For example, measles virus has been used to protect dogs against distemper, and bovine viral diarrhea virus can protect swine against hog cholera (see [Chapter 18](#), [Box 18-2](#)).

Under some circumstances it is possible to use fully virulent organisms for immunization just as the Chinese once did with smallpox. Vaccination against contagious ecthyma of sheep is of this type. Contagious ecthyma (orf) is a viral disease of lambs that causes massive scab formation around the mouth, prevents feeding, and results in a failure to thrive. The disease has little systemic effect. Lambs recover completely within a few weeks and are immune from then on. It is usual to vaccinate lambs by rubbing dried, infected scab material into scratches made in the inner aspect of the thigh. The local infection at this site has no untoward effect on the lambs, and they become solidly immune. Because the vaccinated animals may spread the disease, however, they must be separated from unvaccinated animals for a few weeks.

20.5 MODERN VACCINE TECHNOLOGY

Although both killed and modified live vaccines have been successful in controlling many infectious diseases, there is always a need to make them more effective, cheaper, and safer ([Figure 20-4](#)). The use of modern molecular techniques can produce new and improved vaccines. The U.S. Department of Agriculture (USDA) classifies these vaccines into three categories ([Table 20-1](#)).

20.5.1 Antigens Generated by Gene Cloning (Category I)

Gene cloning can be used to produce large quantities of purified antigen. In this process, DNA coding for an antigen of interest is first isolated. This DNA is then inserted into a bacterium, yeast, or other cell, and the recombinant antigen is expressed. The first successful use of gene cloning to prepare an antigen in this way involved foot-and-mouth disease virus ([Figure 20-5](#)). This virus is extremely simple. The protective antigen (VP1) is well recognized, and the genes that code for

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FIGURE 20-4 A schematic diagram showing some of the different ways in which a virus and its antigens may be treated in order to produce a vaccine.

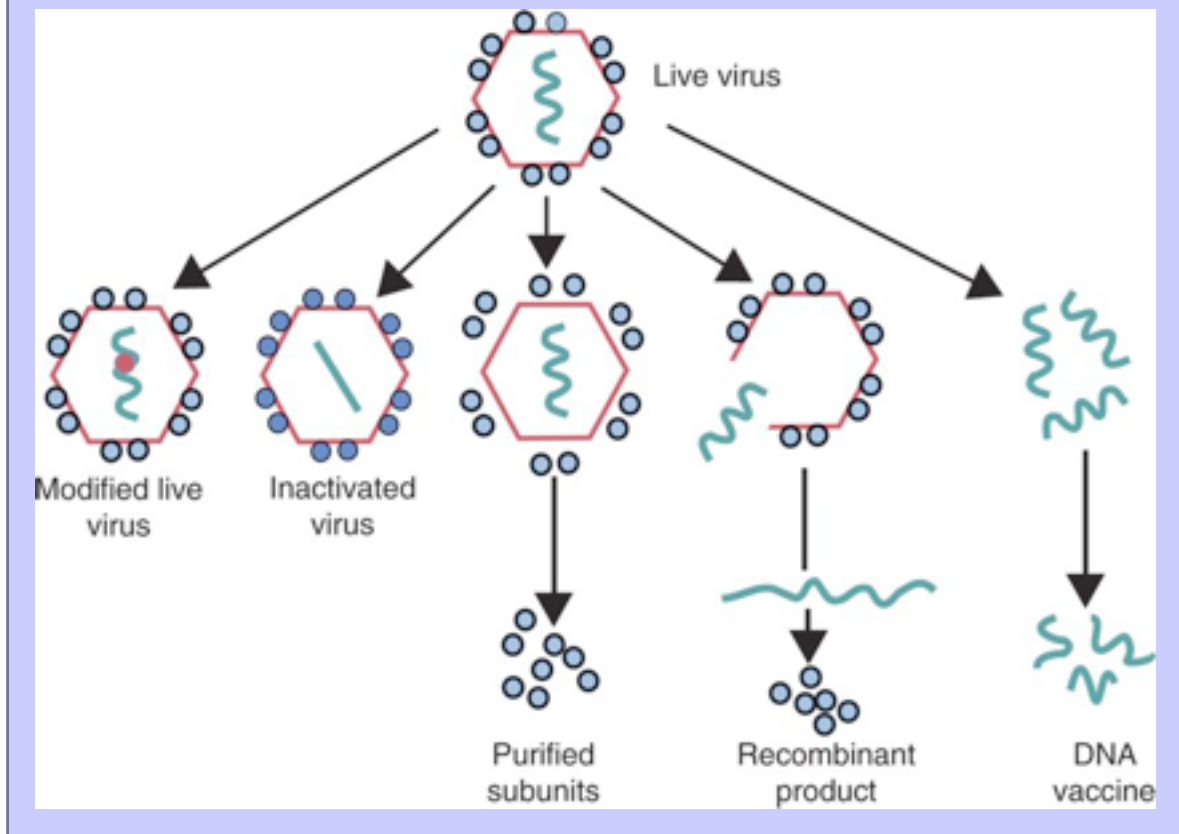
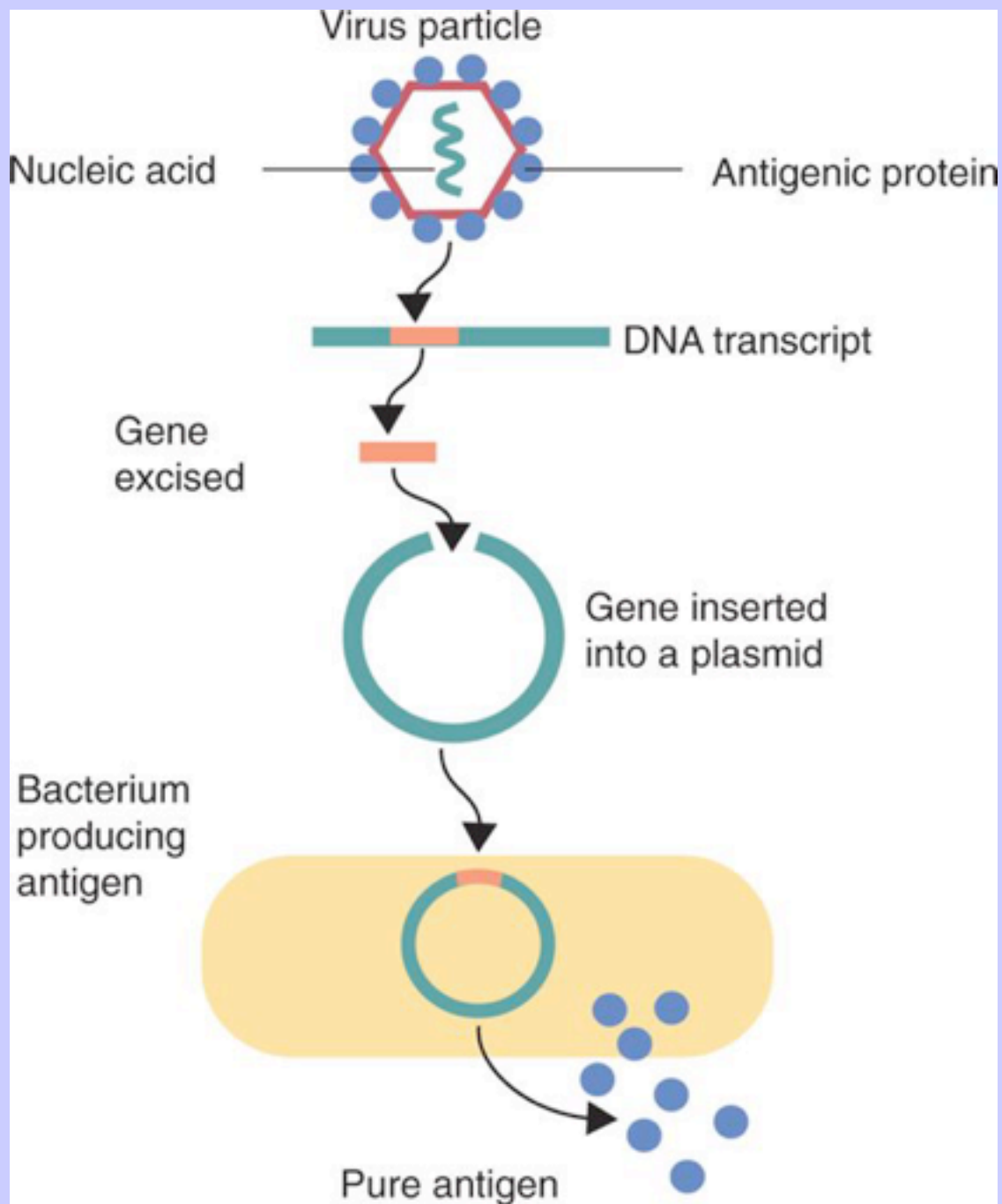


FIGURE 20-5 The production of a recombinant viral protein for use in a vaccine. The gene coding for the viral antigen of interest is cloned into another organism, in this case a bacterium, and expressed and produced in very large quantities.



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this protein have been mapped. The RNA genome of the foot-and-mouth disease virus was isolated and transcribed into DNA by the enzyme reverse transcriptase. The DNA was then carefully cut by restriction endonucleases so that it contained only the gene for VP1. This DNA was then inserted into a plasmid, the plasmid inserted into *E. coli*, and the bacteria grown. The recombinant bacteria synthesized large quantities of VP1 that was harvested, purified, and incorporated into a vaccine. The process is highly efficient since 4×10^7 doses of foot-and-mouth vaccine can be obtained from 10 L of *E. coli* grown to 10^{12} organisms per ml. Unfortunately, the immunity produced is inferior to that produced by killed virus and requires a 1000-fold higher dose to induce comparable protection.

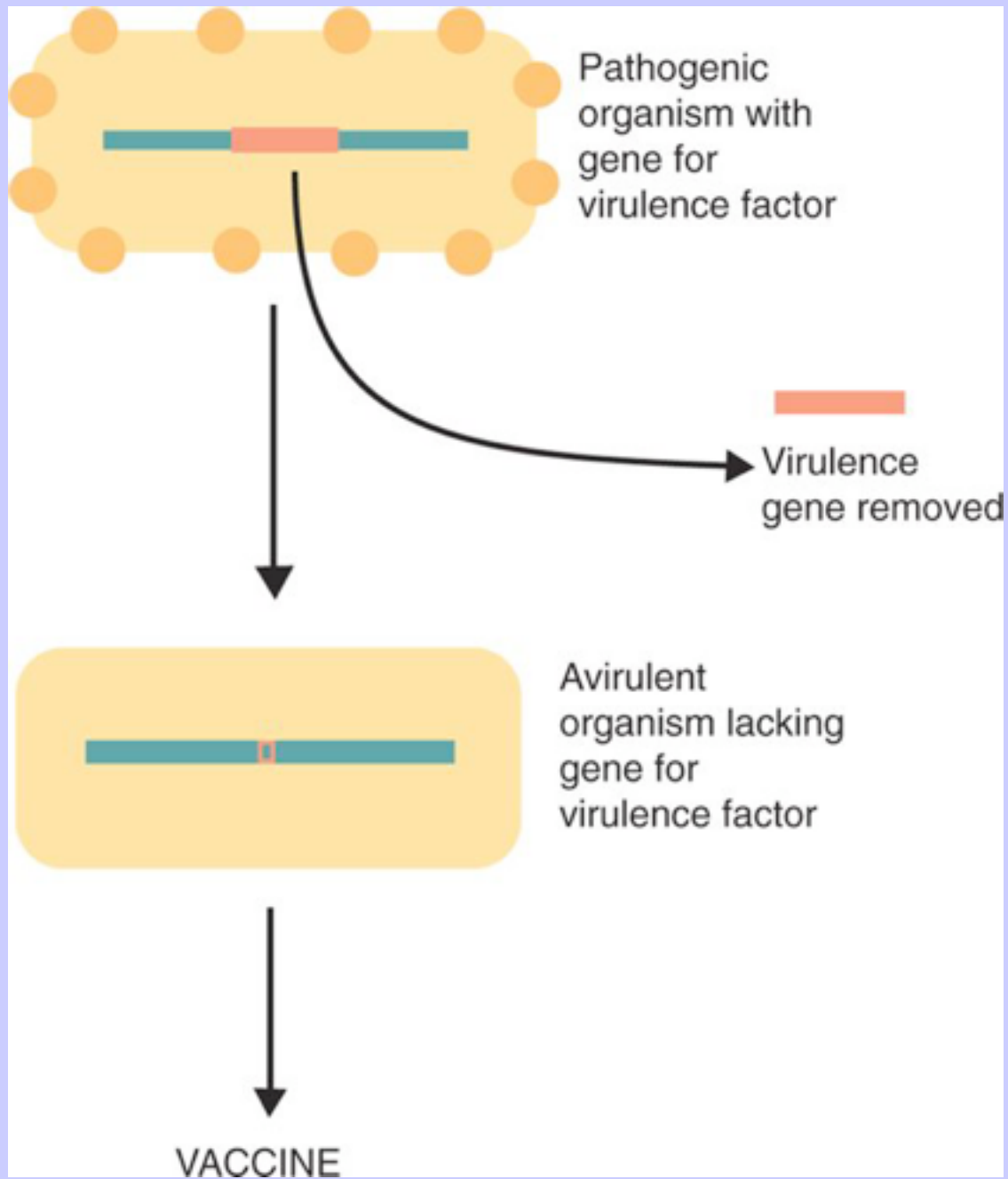
Table 20-1 The USDA Classification of Genetically Engineered Veterinary Biologics

Category	Description
I	Vaccines that contain inactivated recombinant organisms or purified antigens derived from recombinant organisms
II	Vaccines containing live organisms that contain gene deletions or heterologous marker genes
III	Vaccines that contain live expression vectors expressing heterologous genes for immunizing antigens or other stimulants

The first commercially available category I recombinant veterinary vaccine was made against feline leukemia virus (FeLV). The major envelope protein of FeLV, gp70, is the antigen largely responsible for inducing a protective immune response in cats. Thus the gene for gp70 (a glycoprotein of 70 kDa) plus a small portion of a linked protein called p15e (a protein of 15 kDa from the envelope) was isolated and inserted into *E. coli*, which then synthesized large amounts of p70. This recombinant p70 is not glycosylated and has a molecular weight of just over 50 kDa. Once cloned, the recombinant protein is harvested, purified, mixed with a saponin adjuvant, and used as a vaccine.

Another example of a recombinant vaccine is one directed against the Lyme disease agent *Borrelia burgdorferi*. Thus the gene for OspA, the immunodominant outer surface lipoprotein of *B. burgdorferi*, was cloned into *E. coli*. The recombinant protein expressed by the *E. coli* is purified and used as a vaccine when combined with adjuvant. This vaccine is unique since ticks feeding on immunized animals ingest the antibody. The antibodies then kill the bacteria within the tick midgut and prevent their dissemination to the salivary glands. They thus prevent transmission by the vector.

FIGURE 20-6 The production of an attenuated virus by removal of a gene required for virulence. Genes coding for major antigens detected by serological techniques can also be removed, ensuring that vaccinated animals can be distinguished from naturally infected ones.



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Rather than cloning the gene of interest in a microorganism, it is possible to clone antigen genes in plants. This has been successfully achieved for viruses such as transmissible gastroenteritis and Newcastle disease. The plants employed include tobacco, potato, and corn. In some cases these plants contain very high concentrations of antigen and so can simply be fed to animals. Although results have been mixed, a tobacco plant-based Newcastle disease vaccine has been licensed in the United States. It is unclear just how these vaccines will be best employed in animals.

Gene cloning techniques are useful in any situation in which pure protein antigens need to be synthesized in large quantities. Unfortunately, pure proteins are often poor antigens because they are not effectively delivered to antigen-sensitive cells and they may not be correctly folded. In addition, they may be inefficient antigens because of MHC restriction. An alternative method of delivering a recombinant antigen is to clone the gene of interest into an attenuated living carrier organism.

20.5.2 Genetically Attenuated Organisms (Category II)

Attenuation by prolonged tissue culture can be considered as a primitive form of genetic engineering. The desired result is the development of a strain of organism that cannot cause disease. This may be difficult to achieve, and reversion to virulence is an ever-present risk. Molecular genetic techniques, however, make it possible to modify the genes of an organism so that it becomes irreversibly attenuated. The USDA classifies these as category II vaccines. They are now available against the herpesvirus that causes pseudorabies in swine. The enzyme, thymidine kinase (TK), is required by herpesviruses to replicate in nondividing cells such as neurons. Viruses from which the TK gene has been removed can infect nerve cells but cannot replicate and therefore cannot cause disease ([Figure 20-6](#)). As a result, these vaccines not only confer effective protection, but also block cell invasion by virulent pseudorabies viruses and so prevent the development of a persistent carrier state.

Genetic manipulation can also be used to make “marker vaccines.” Thus, for example, pseudorabies virus synthesizes two glycoprotein antigens called gX and gI. These are potent antigens, yet neither is essential for viral growth or virulence. They are expressed by all field isolates of this virus. Animals infected with the field virus will make antibodies to gX and gI. It has proved possible to construct an attenuated pseudo-rabies virus that lacks either gX or gI and can be used as a vaccine. Vaccinated pigs will not make antibodies to gX or gI, but naturally infected pigs will. The vaccine will not cause positive serological reactions in ELISA tests for gX or gI, and the presence of antibodies to gX and gI in a pig is evidence that the animal has been exposed to field strains of pseudorabies virus. This type of vaccine, called a DIVA vaccine (*differentiate infected from vaccinated animals*), should assist in eradicating specific infectious diseases much more economically and rapidly than conventional methods.

20.5.3 Live Recombinant Organisms (Category III)

Genes coding for protein antigens can be cloned directly into a variety of organisms. Instead of being purified, the recombinant organism itself may then be used as a vaccine. The USDA classifies these as category III vaccines ([Figure 20-7](#)). Experimental recombinant vaccines have used adenoviruses, herpesviruses, and bacteria such as BCG or *Salmonella*, but the organisms that have been most widely employed for this purpose are poxviruses such as vaccinia, fowlpox, or canarypox. These viruses are easy to administer by dermal scratching or by ingestion. They have a large genome that makes it relatively easy to insert a new gene (up to 10% of its genome can be replaced by foreign DNA), and they can express high levels of the new antigen. Moreover, these

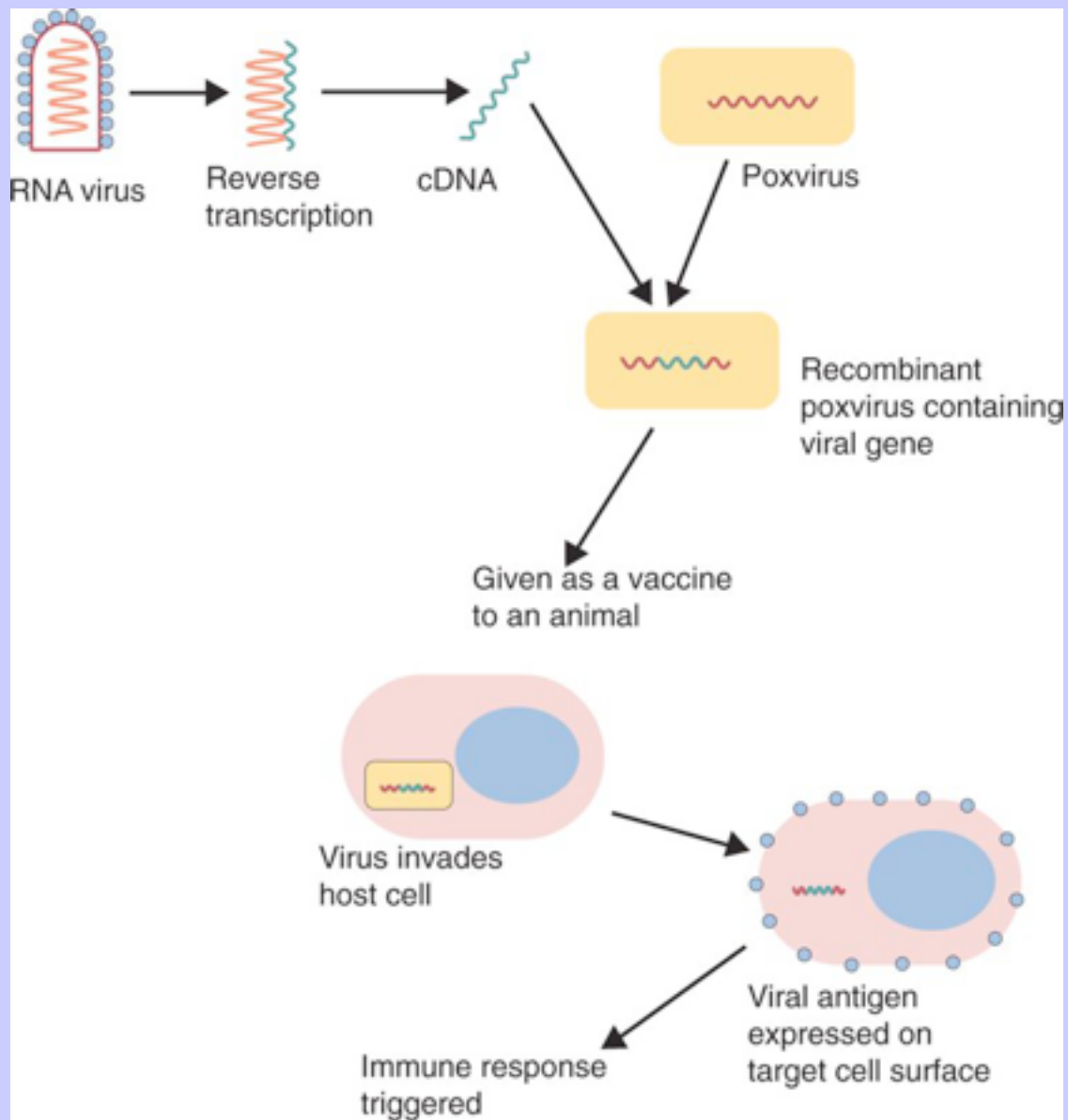
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recombinant proteins undergo appropriate processing steps including glycosylation and membrane transport within the poxvirus.

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FIGURE 20-7 The production of a vaccinia recombinant vaccine. Vaccinia is selected because it has room to spare in its genome, and it is easy to administer to an animal. Thus rabies-vaccinia recombinants can be given orally.



A good example of such a vaccine is the vaccinia virus recombinant, which contains the gene for the rabies envelope glycoprotein or G protein. The G glycoprotein forms the characteristic spikes on the surface of rabies virus. This glycoprotein is the only rabies antigen capable of inducing virus-neutralizing antibody and conferring

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protection against rabies. Infection with this rabies-vaccinia recombinant results in the production of antibodies to the G protein and the development of immunity. This vaccine has been successfully used as an oral vaccine administered to wild carnivores in bait. This form of the vaccine can be distributed by dropping from aircraft. Thus in Belgium, oral rabies vaccine dropped from the air effectively terminated fox rabies that was spreading through the Ardennes ([Figure 20-8](#)). It has been used in Ontario to prevent the spread of fox rabies, in New Jersey to prevent the spread of raccoon rabies, and in Texas to block the spread of coyote rabies. For example, since 1995, 17.5 million doses of recombinant rabies vaccine have been air dropped over 255,500 square miles (661,745 sq km) over Texas with great success.

Effective category III vaccines have also been developed for rinderpest; these consist of a vaccinia or capripox vector containing the hemagglutinin (H) or fusion (F) genes of rinderpest virus. The recombinant capripox vaccine has the benefit of protecting cattle against both rinderpest and lumpy skin disease. The vaccinia virus can be further attenuated by inactivation of its TK and hemagglutinin genes. This has the advantage that it does not cause skin lesions in vaccinated animals. Swinepox has been tested as a potential vector for pseudorabies in pigs.

Another example of a category III vaccine involves the use of a yellow fever viral chimera to protect against West Nile virus. This technology uses the capsid and nonstructural genes of the attenuated yellow fever vaccine strain 17D to deliver the envelope genes of other flaviviruses such as West Nile virus. The resulting virus is a yellow fever/West Nile virus chimera that is much less neuroinvasive and hence much safer than either of the parent viruses. The margin of safety can be increased even further by introducing targeted point mutations into the envelope genes.

The first category III vaccine approved by the USDA was against the Newcastle disease virus. The carrier organism is a fowlpox virus, into which Newcastle disease HA and F genes have been incorporated. It has the benefit of conferring immunity against fowlpox as well. Canarypox-vectored vaccines are successfully employed in dogs and cats.

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FIGURE 20-8 The geographical distribution of cases of wild animal rabies in Belgium. **A**, In 1989. **B**, In 1992 and 1993, following the introduction of an oral rabies-vaccinia recombinant vaccine. A remarkable example of the effectiveness of a recombinant vaccine. (From Brochier B, Boulanger D, Costy F, Pastoret PP: *Vaccine* 12:1368-1371, 1994.)

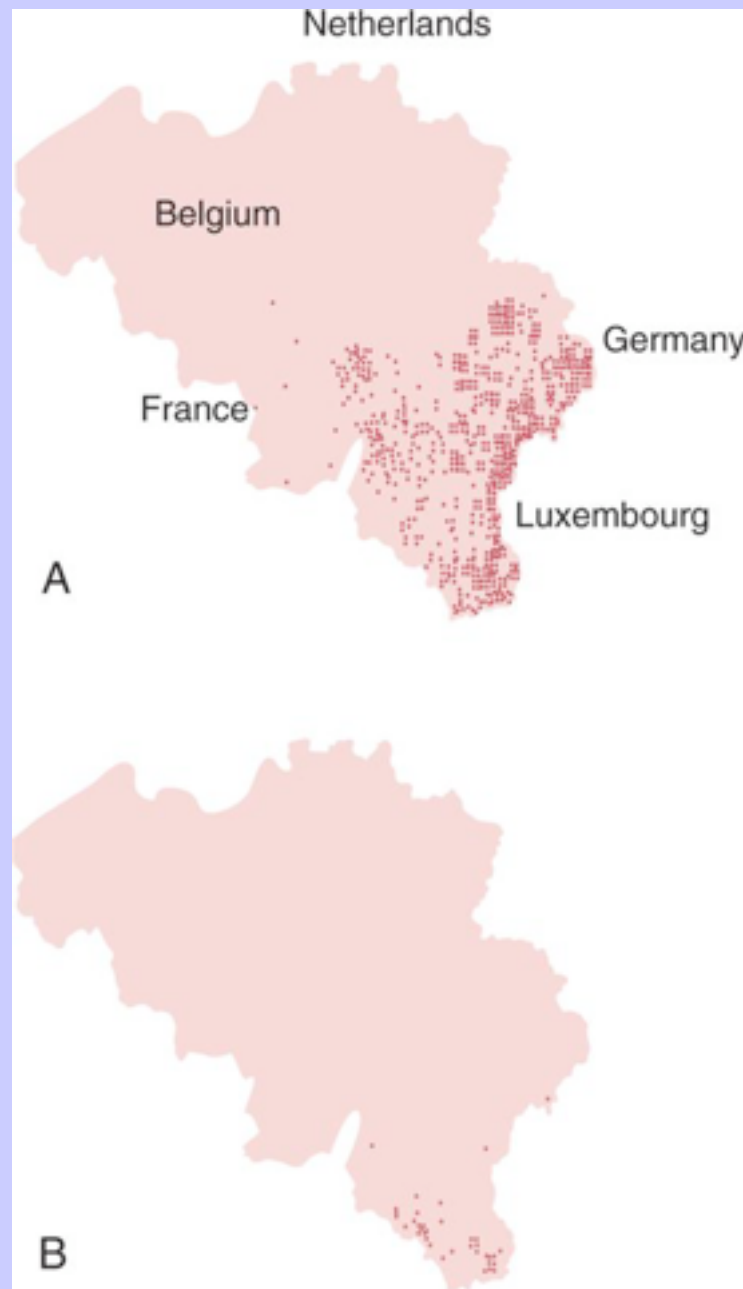


FIGURE 20-9 The mechanism by which polynucleotide vaccines can work. The DNA that enters a cell is functional and can code for endogenous antigens.

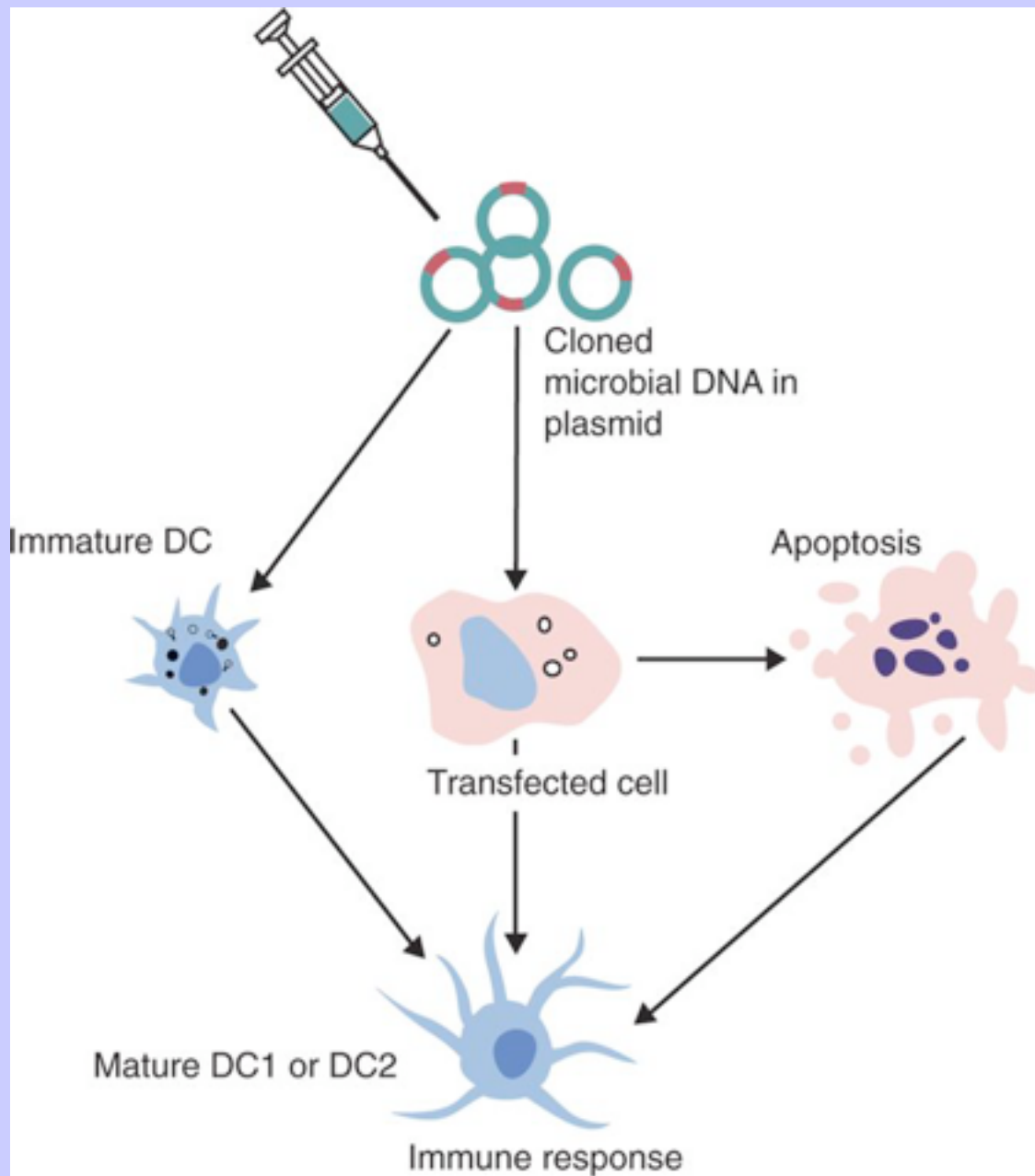
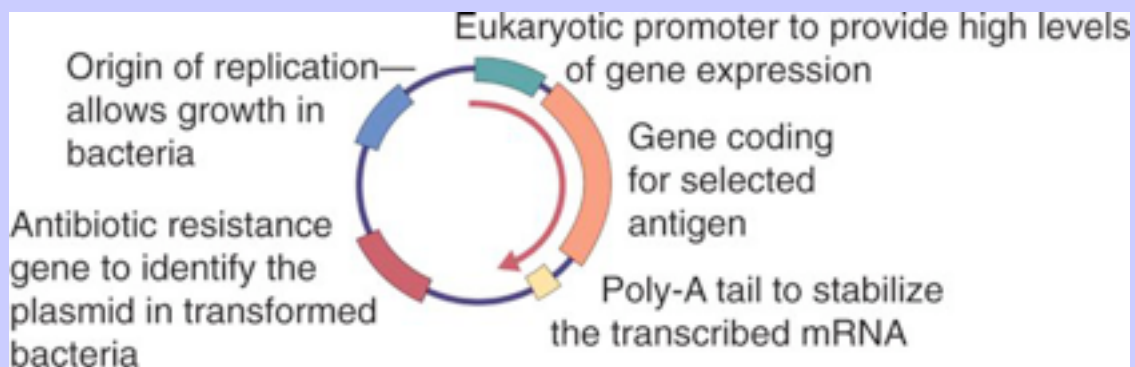


FIGURE 20-10 The structure of a typical DNA plasmid used for vaccination purposes. In addition to coding for the antigen in question, the plasmid carries an antibiotic resistance marker so that its fate may be traced.



20.5.4 Polynucleotide Vaccines

Another method of vaccination involves injection, not of a protein antigen but of DNA that encodes foreign antigens. For example, the DNA coding for a vaccine antigen can be inserted into a bacterial plasmid, a piece of circular DNA that acts as a vector (Figure 20-9). The vaccine antigen gene is placed under the control of a strong mammalian promoter sequence. When the genetically engineered plasmid is injected intramuscularly into an animal, it is taken up by host cells. The DNA is then transcribed into mRNA and translated into endogenous vaccine protein (Figure 20-10). The plasmid, unlike viral vectors, cannot replicate in mammalian cells. Experience has shown that plasmid incorporation is enhanced by the use of some “adjuvants.” These may include lipid complexes, microcapsules, or nonionic copolymers. Aluminum phosphate seems especially effective in improving vaccine efficiency. Transfected host cells express the vaccine protein in association with MHC class I molecules, as do other endogenous antigens. This can lead to the development of not only neutralizing antibodies but also, since the antigen is endogenous, of cytotoxic T cells. Expressed antigens will have authentic tertiary structure and posttranslational modifications such as glycosylation. The immune response is also enhanced since the bacterial DNA contains unmethylated CpG motifs that are recognized by toll-like receptor 9 (TLR9) and stimulate the activation of dendritic cells. These in turn promote a strong Th1 response with resulting immunity to viruses or intracellular bacteria. This type of vaccine is used successfully to protect horses against West Nile virus infection. The commercial vaccine consists of a plasmid vector engineered to express high levels of the virus envelope (E) and premembrane (prM) proteins. In addition the plasmid contains gene promoters and marker genes. Upon injection together with a biodegradable oil adjuvant, this plasmid enters cells and causes them to express high levels of the viral protein. Two doses are administered 3 to 4 weeks apart. This approach has also been applied experimentally to produce vaccines against bovine herpes, avian influenza, lymphocytic choriomeningitis, canine and feline rabies, canine parvovirus, bovine viral diarrhea, feline immunodeficiency virus, FeLV, pseudorabies, influenza, foot-and-mouth disease virus, bovine herpesvirus 1, and Newcastle disease. Although theoretically producing a response similar to that induced by attenuated live vaccines, these nucleic acid vaccines are ideally suited to prepare vaccines against organisms that are difficult or dangerous to grow in the laboratory. DNA vaccines cannot, of course, be used to induce immunity to polysaccharide antigens.

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Some DNA vaccines appear to be able to induce immunity even in the presence of very high titers of maternal antibody. Although the maternal antibodies block serological responses, the development of strong memory responses is not impaired.

Polynucleotide vaccines must be delivered inside target cells. This can be done by intramuscular injection or by “shooting” the DNA plasmids directly through the skin adsorbed onto microscopic gold beads fired by a “gene gun.” Although intramuscular injection is very inefficient because the transfection rate is low (about 1% to 5% of myofibrils in the vicinity of an intramuscular injection site), the expression can persist for at least 2 months. The gene products are either treated as endogenous antigens and displayed on the cell surface or secreted and presented to antigen-processing cells. This processed antigen thus preferentially stimulates a Th1 response associated with interferon- γ (IFN- γ) production. The use of a gene gun is more efficient than injection since some of this DNA is taken up by the animal's dendritic cells directly and it minimizes degradation. By bypassing TLR9, this material appears to preferentially stimulate a Th2 response. This is, of course, of major practical significance. Viral DNA in eye drops can induce an IgA response in the tears and bile of recipients.

Immunization with purified DNA in this way allows presentation of viral antigens in their native form, which are synthesized in the same way as antigens during a viral infection. This is a great improvement over the use of recombinant proteins, which have proven difficult to create in the correct conformation. Another advantage is that it is possible to select only the genes for the antigen of interest rather than using a complex carrier organism with its own large gene pool and antigenic mass.

As far as safety is concerned, one theoretical disadvantage is the potential for the vaccine DNA to integrate into the host genome and possibly activate oncogenes or inhibit tumor suppressor genes. Experience suggests that this is a low risk. The presence of an antibiotic-resistant gene in these plasmids also runs the risk of transferring this resistance to bacteria. This can be avoided by the use of other markers. Adding cytokines or cytokine genes such as those for IL-3 may also result in improved responses.

20.5.4.1

Prime-Boost Strategies

It has long been normal practice to use exactly the same vaccine for boosting an immune response as was employed when first priming an animal. This approach has many advantages, not the least of which is simplicity in manufacturing and regulating vaccine production. There is, however, no reason why different forms of a vaccine may not be used for priming and for boosting. This approach is known as a prime-boost strategy. Under some circumstances this may result in significantly improved vaccine effectiveness. The prime-boost approach is somewhat empirical and researchers may simply test numerous vaccine combinations to determine which combination yields the best results. Prime-boosting has been most widely investigated in attempts to improve the effectiveness of DNA vaccines. Combinations usually involve priming with a DNA vaccine but either boosting with another DNA vaccine, perhaps in another vector, or boosting with recombinant protein antigens.

20.5.5

Synthetic Peptides

Now that complete bacterial genomes are available, it is possible to identify potential vaccine antigens by computer analysis. This analysis can rapidly identify novel potential vaccine candidates—a process called reverse vaccinology. The procedures involved include a complete sequencing of the antigens of interest, followed by identification of their important epitopes. These epitopes may be predicted by the use of computer models of the protein or by the use of mono-clonal antibodies to identify critical protective components. Once

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identified, the protective epitopes may be chemically synthesized. Experimental synthetic vaccines have been developed against hepatitis B, diphtheria toxin, foot-and-mouth-disease virus, canine parvovirus, and influenza A, and they provoke some protective immunity.

20.6

ADJUVANTS

To maximize the effectiveness of vaccines, especially those containing poorly antigenic-killed organisms or highly purified antigens, it has been common practice to add substances called adjuvants to the antigen. Adjuvants can greatly enhance the body's response to vaccines, they may permit reductions in the amount of antigen injected or the numbers of doses administered, and they are essential if long-term memory is to be established to soluble antigens. The mechanisms of adjuvant action are only poorly understood, a problem that has hampered rational adjuvant development and which has made adjuvant selection somewhat empirical. In general, however, adjuvants work through one of three mechanisms ([Table 20-2](#)). Depot adjuvants simply protect antigens from rapid degradation and so prolong immune responses. A second group consists of particles that effectively deliver antigen to antigen-presenting cells and so enhance anti-gen presentation. A third group—immunostimulatory adjuvants—consists of molecules that enhance cytokine production and selectively stimulate Th1 or Th2 responses by providing appropriate co-stimulation.

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Table 20-2 Some Common Adjuvants

Type	Adjuvant	Mode of Action
Depot adjuvants	Aluminum phosphate	Slow-release antigen depot
	Aluminum hydroxide	Slow-release antigen depot
	Alum	Slow-release antigen depot
	Freund's incomplete adjuvant	Slow-release antigen depot
Microbial adjuvants	Anaerobic corynebacteria	Macrophage stimulator
	BCG	Macrophage stimulator
	Muramyl dipeptide	Macrophage stimulator
	<i>Bordetella pertussis</i>	Lymphocyte stimulator
	Lipopolysaccharide	Macrophage stimulator
Immune stimulators	Saponin	Stimulates antigen processing
	Lysolecithin	Stimulates antigen processing
	Pluronic detergents	Stimulates antigen processing
	Acemannan	Macrophage stimulator
	Glucans	Macrophage stimulator
	Dextran sulfate	Macrophage stimulator
Delivery systems	Liposomes	Stimulates antigen processing
	ISCOMs	Stimulates antigen processing
	Microparticles	Stimulates antigen processing
Mixed adjuvants	Freund's complete adjuvant	Water-in-oil emulsion plus <i>Mycobacterium</i>

20.6.1 Depot Adjuvants

Some adjuvants simply delay the elimination of antigens and thus permit an immune response to last longer. The immune system, being antigen driven, responds to the presence of antigen and terminates once antigen is eliminated. The rate of antigen elimination can be slowed by mixing it with an insoluble, slowly degraded adjuvant. Examples of depot-forming adjuvants include aluminum salts, such as aluminum hydroxide, aluminum phosphate, and aluminum potassium sulfate (alum), as well as calcium phosphate ([Figure 20-11](#)). When antigen is mixed with one of these salts and injected into an animal, a macrophage-rich granuloma forms in the tissues. The antigen within this granuloma slowly leaks into the body and so provides a prolonged antigenic stimulus. Antigens that normally persist for only a few days may be retained in the body for several weeks by this technique. These depot adjuvants influence only the primary immune response and have little effect on secondary immune responses. Aluminum-based adjuvants also have the disadvantage that while promoting antibody responses, they have little stimulatory effect on cell-mediated responses.

An alternative method of forming a depot is to incorporate the antigen in a water-in-oil emulsion (called Freund's incomplete adjuvant). The light mineral oil stimulates a local, chronic inflammatory response, and as a result, a

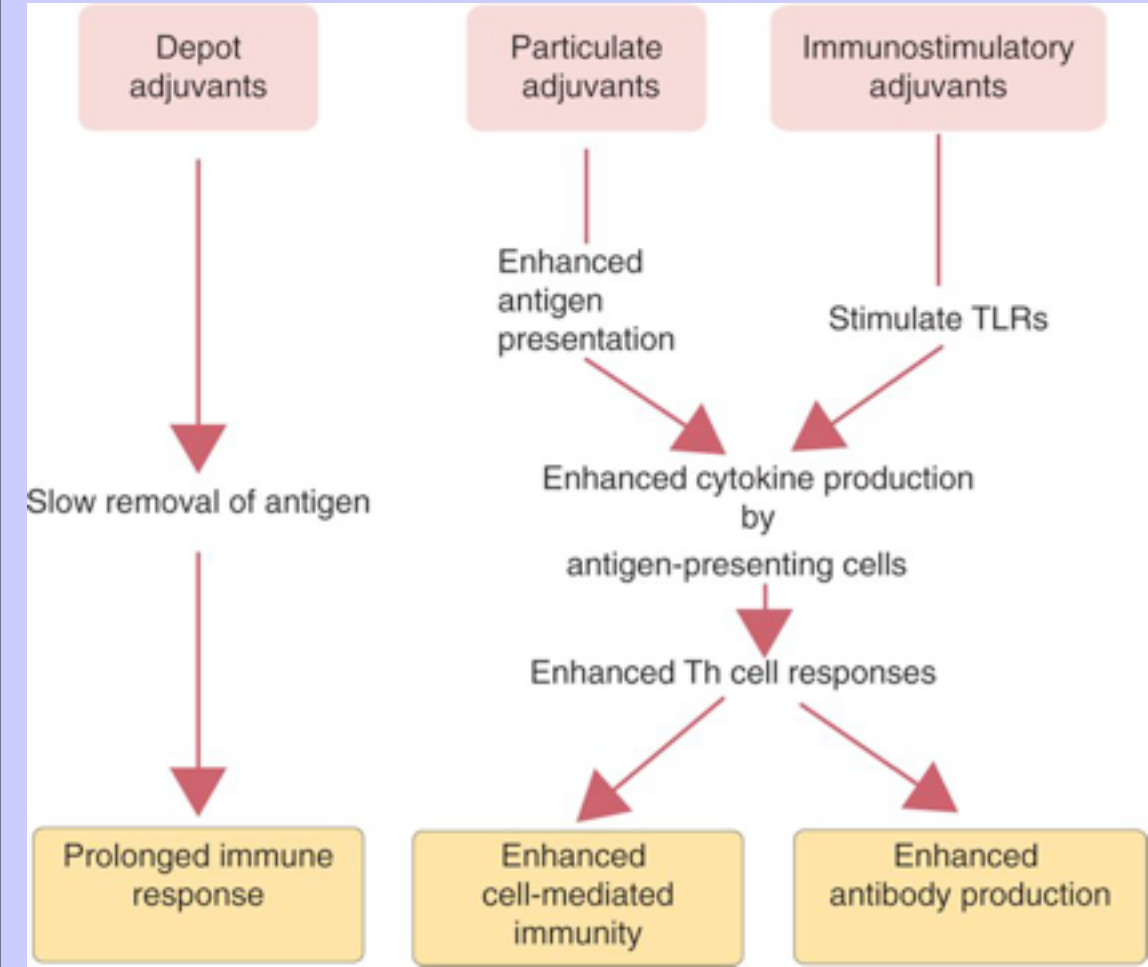
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granuloma or abscess forms around the site of the inoculum. The antigen is slowly leached from the aqueous phase of the emulsion. These depot adjuvants may cause significant tissue irritation and destruction. Mineral oils are especially irritating. Nonmineral oils, although less irritating, are also less effective. Tissue damage induced by adjuvants may also promote immune responses since the alarmins generated by inflammation and cellular necrosis stimulate both dendritic cells and macrophages. Significant irritant activity of adjuvants is not, however, acceptable in modern vaccines, and strenuous

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FIGURE 20-11 The three major groups of adjuvants and the ways in which these may act to enhance immune responses triggered by vaccine antigens.



attempts are being made to reduce this irritation while retaining adjuvant effectiveness.

20.6.2

Particulate Adjuvants

The immune system can generally trap and process particles such as bacteria or other microorganisms much more efficiently than soluble antigens. As a result, many attempts have been made to incorporate antigens into readily phagocytosable particles. These adjuvants include emulsions, microparticles, immune-stimulating

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complexes (ISCOMs), and liposomes, and all are designed to deliver antigen efficiently to antigen-presenting cells. They commonly are of similar size to bacteria and are readily endocytosed by antigen-presenting cells. Biodegradable microparticles incorporating antigens are usually designed to be readily phagocytosed. Liposomes are lipid-based synthetic microparticles containing encapsulated antigens that are readily trapped and processed yet are also protected from rapid degradation. ISCOMs, described below, are complex lipid-based microparticles. All of these particulate adjuvants may be made more potent by incorporating microbial immunostimulants. They are not yet widely employed in veterinary vaccines.

20.6.3 Immunostimulatory Adjuvants

Immunostimulatory adjuvants exert their effects by promoting cytokine production. Many of them are complex microbial products that often represent pathogen-associated molecular patterns. As a result, they activate dendritic cells and macrophages through TLRs and stimulate the secretion of key cytokines such as IL-1 and IL-12. These cytokines in turn promote helper T cell responses and drive and focus the acquired immune responses. Depending on the specific microbial product, they may enhance either Th1 or Th2 responses.

Commonly employed microbial immunostimulants include lipopolysaccharides (or their derivatives). These enhance antibody formation if given at about the same time as the antigen. They have no effect on cell-mediated responses. But they can break tolerance, and they have a general immunostimulatory activity, which is reflected in a nonspecific resistance to bacterial infections. Killed anaerobic corynebacteria, especially *Propionibacterium acnes*, have a similar effect. When used as adjuvants, these bacteria enhance antibacterial and antitumor activity. *Bordetella pertussis*, the cause of whooping cough, also prolongs and enhances immunological memory and stimulates macrophage activity. *B. pertussis* may selectively enhance Th2 responses and IgE production. Microbial CpG DNA also appears to be a potent immunostimulatory adjuvant for Th1 responses.

Another group of immunostimulatory adjuvants is the saponins (triterpene glycosides), derived from the bark of the soapbark tree (*Quillaja saponaria*). Saponins have both toxic and adjuvant activities, although fractionation can separate those that have potent adjuvant activity and are relatively nontoxic. Saponin-based adjuvants may selectively stimulate Th1 activity, since they direct antigens into endogenous processing pathways and enhance co-stimulatory activity. A purified saponin is used as an adjuvant in a recombinant feline leukemia vaccine. Toxic saponin mixtures are used in anthrax vaccines, where they destroy tissue at the site of injection so that the anthrax spores may germinate. Saponin is also employed as an adjuvant for foot-and-mouth disease vaccines. DEAE dextran may be an effective substitute for saponins in some vaccines.

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20.6.4 Combined Adjuvants

Very powerful adjuvants can be constructed by combining a particulate or depot adjuvant with an immunostimulatory agent. For example, an oil-based depot adjuvant can be mixed with killed *Mycobacterium tuberculosis* incorporated into the water-in-oil emulsion. The mixture is called Freund's complete adjuvant (FCA). Not only does FCA form a depot, but the tubercle bacilli contain muramyl dipeptide (*n*-acetylmuramyl-l-alanyl-d-isoglutamine), a molecule that activates macrophages and dendritic cells through nucleotide-binding oligomerization domain 2 (NOD2). FCA works best when given subcutaneously or intradermally and when the antigen dose is relatively low. FCA promotes IgG production over IgM. It inhibits tolerance induction, favors delayed hypersensitivity reactions, accelerates graft rejection, and promotes resistance to tumors. FCA can be used to induce some experimental autoimmune diseases, such as experimental allergic encephalitis and thyroiditis (see [Chapter 31](#)). It also stimulates macrophage conversion to M1 cells, thus promoting their phagocytic and cytotoxic activities.

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Use of oil-based adjuvants in animals intended for human consumption is problematic, since the oil may spoil the meat. Use of FCA is unacceptable in cattle, not only because of the mineral oil but also because the mycobacteria in the adjuvant may induce a positive skin reaction to tuberculin, a critical drawback in any area in which tuberculosis is controlled by skin testing. FCA is highly toxic in dogs and cats.

ISCOMs are stable complexes containing cholesterol, phospholipid, saponin, and antigen. Micelles may be constructed using protein antigens and a matrix of a saponin mixture called Quil A. These ISCOMs are effective adjuvants with few adverse side effects. They are highly effective in targeting antigens to the professional antigen-processing cells, while the saponin activates these cells and so promotes cytokine production and the expression of co-stimulatory molecules. Depending on the antigen employed, ISCOMs can stimulate either Th1 or Th2 responses.

Given that many adjuvants act by stimulating cytokine production, it is logical that some cytokines may be effective adjuvants. Most cytokines tested in this way have unacceptable toxicity, although IL-12 appears to be especially effective in this regard, since as a Th1 cell stimulator, it enhances IFN- γ production. IL-3 appears to potentiate some DNA vaccines.

By far the most widely employed adjuvants in commercial veterinary vaccines are the depot adjuvants such as aluminum hydroxide, aluminum phosphate, or aluminum potassium sulfate (alum). These adjuvants are produced in the form of a colloidal suspension to which the antigenic material is adsorbed. They are stable on storage, and although they produce a small local granuloma on inoculation, they do not track between muscles or make large parts of the carcass unsuitable for consumption.

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21 CHAPTER 21 The Use of Vaccines

21.1 KEY POINTS

- Vaccine usage should be determined by a careful assessment of the relative risks and benefits to an animal.
- Vaccines should be administered only in the doses and by the routes recommended by the manufacturer.
- Vaccines should not be given more often than necessary to provide effective protection.
- On occasion vaccines may cause adverse effects in animals. These are often mild but may be life-threatening, including allergic reactions and the development of cancer in cats.

Although the principles of vaccination have been known for many years, vaccines and vaccination procedures continue to evolve as we seek to improve both efficacy and safety. When first developed, many vaccines were of limited efficacy and induced severe adverse effects, although these effects were considered acceptable when measured against the risks of acquiring disease. The vaccination protocols developed at that time reflected the short duration of immunity induced by these vaccines. Ongoing developments in vaccine design and production have resulted in great improvements in both vaccine safety and effectiveness. These improvements have led to a reassessment of the relative risks and benefits of vaccination and have resulted in changes in vaccination protocols. Vaccination is not always an innocuous procedure and may occasionally cause sickness or death. For this reason, the use of any vaccine should be accompanied by a risk-benefit analysis of the need for administration of a vaccine conducted by the veterinarian in consultation with an animal's owner. Vaccination protocols should be customized to each animal, giving due consideration to the seriousness and zoonotic potential of the agent, the animal's exposure risk, and any legal requirements relating to vaccination.

The two major factors that determine vaccine usage are safety and efficacy. We must always be sure that the risks of vaccination do not exceed those associated with the chance of contracting the disease itself. Thus it may be inappropriate to use a vaccine against a disease that is rare, is readily treated by other means, or is of little clinical significance. In addition, because the detection of antibodies is a common diagnostic procedure, unnecessary use of vaccines may complicate diagnosis based on serology and perhaps make eradication of a disease impossible. Because of this, the decision to use vaccines for the control of any disease must be based not only on the degree of risk associated with the disease but also on the availability of superior control or treatment procedures.

The second major consideration is vaccine efficacy. Vaccines are not always effective. Thus in some diseases, such as equine infectious anemia, Aleutian disease in mink, and African swine fever, poor or no protective immunity can be induced even with the best vaccines. In other diseases, such as foot-and-mouth disease in pigs, the immune response is transient and relatively ineffective, so that successful vaccination is difficult to achieve.

As a result of these considerations, some investigators have recommended that animal vaccines be divided into categories based on their importance. The first category consists of essential (or core) vaccines—those vaccines that are required because they protect against common, dangerous diseases so that a failure to use them would place an animal at significant risk of disease or death. The second category consists of optional (or noncore) vaccines. These are directed against diseases for which the risks associated with not vaccinating may be low. In many cases, risks from these diseases are determined by the location or lifestyle of an animal. The use of these optional vaccines would be determined by a veterinarian on the basis of exposure risk. A third category consists of those vaccines that may have no application in routine vaccination but might be used under very special circumstances. These are vaccines

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that would be directed against diseases of little clinical significance or vaccines whose risks do not significantly outweigh their benefits. Of course, all vaccine usage should be conducted on the basis of informed consent. An animal's owner should be made aware of the risks and benefits involved before seeking approval to vaccinate.

When vaccines are used to control disease in a population of animals rather than in individuals, the concept of herd immunity should also be considered. This herd immunity is the resistance of an entire group of animals to a disease as a result of the presence, in that group, of a proportion of immune animals. Herd immunity reduces the probability of a susceptible animal meeting an infected one and thus slows or terminates the spread of disease. If it is acceptable to lose individual animals from disease while preventing epizootics, it may be possible to do this by vaccinating only a proportion of the population.

21.2

ADMINISTRATION OF VACCINES

Most vaccines are administered by injection. All such vaccines should be injected carefully and with due regard for the anatomy of the animal. Thus care must be taken not to injure or introduce infection into any animal. All needles used must be clean and sharp. Dirty or dull needles can cause tissue damage and infection at the injection site. The skin at the injection site must be clean and dry, although excessive use of alcohol should be avoided. Vaccines are provided in a standard dose, and this dose should not be divided to account for an animal's size. Doses are not formulated to account for body weight or age. There must be a sufficient amount of an antigen to trigger the cells of the immune system and provoke an immune response. This amount is not related to body size. (Unfortunately, the risk of an adverse event occurring is increased in small animals, so it may be necessary to make some adjustment in dose for safety reasons.) Vaccination by subcutaneous or intramuscular injection is the simplest and most common method of vaccine administration. This approach is obviously excellent for small numbers of animals and for diseases in which systemic immunity is important. In some diseases, however, systemic immunity is not as important as local immunity, and it is perhaps more appropriate to administer the vaccine at the site of potential invasions. Therefore intranasal vaccines are available for infectious bovine rhinotracheitis of cattle; for *Streptococcus equi* infections in horses; for feline rhinotracheitis, *Bordetella bronchiseptica*, coronavirus, and calicivirus infections; for canine parainfluenza and *Bordetella*; and for infectious bronchitis and Newcastle disease in poultry. Unfortunately, these methods of administration require that each animal be dealt with on an individual basis. When animal numbers are large, other methods must be employed. For example, aerosolization of vaccines enables them to be inhaled by all the animals in a group. This technique is employed in vaccinating against canine distemper and mink enteritis on mink ranches and against Newcastle disease in poultry. Alternatively, the vaccine may be put in the feed or drinking water, as is done with *Erysipelothrix rhusiopathiae* vaccines in pigs and against Newcastle disease, infectious laryngotracheitis, and avian encephalomyelitis in poultry.

Vaccination is now the most important method of preventing infectious diseases in farmed fish, in which it may significantly reduce mortality. Most commercial vaccines consist of inactivated products that are administered either by intraperitoneal injection or, preferably, by immersing the fish in a dilute antigen solution. Immersion results in the antigen being deposited on mucosal surfaces such as the gills or oral cavity, and some may be swallowed.

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21.2.1

Multiple-Antigen Vaccines

For convenience, it has become common to employ mixtures of organisms within single vaccines. For respiratory diseases of cattle, for example, vaccines are available that contain infectious bovine rhinotracheitis (BHV-1), bovine viral diarrhea (BVD), parainfluenza 3 (P13), and even *Mannheimia haemolytica*. Dogs may be given vaccines containing all of the following organisms: canine distemper virus, canine adenovirus-1, canine adenovirus-2, canine parvovirus-2, canine parainfluenza virus, leptospira bacterin, and rabies vaccine. These

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mixtures may be used when exact diagnosis is not possible and may protect animals against several infectious agents with economy of effort. However, it can also be wasteful to use vaccines against organisms that may not be causing problems. When different antigens in a mixture are inoculated simultaneously, competition occurs between antigens. Manufacturers of multiple-antigen vaccines take this into account and modify their mixtures accordingly. Vaccines should never be mixed indiscriminately, since one component may dominate the mixture or interfere with the response to the other components.

Some veterinarians have questioned whether the use of complex vaccine mixtures leads to less than satisfactory protection or increases the risk for adverse side effects. The suggestion that these multiple-antigen vaccines can overload the immune system is unfounded, nor is there any evidence to support the contention that the risk for adverse effects increases disproportionately when more components are added to vaccines. Certainly such vaccines should be tested to ensure that all components induce a satisfactory response. Licensed vaccines provided by a reputable manufacturer will generally provide satisfactory protection against all components.

Studies on mink receiving repeated multiple antigen vaccines have suggested that vaccinated animals may have significantly more immunoglobulins deposited in their glomeruli than animals receiving monovalent vaccine. This may be due to immune complex deposition or perhaps to individual components within the multivalent product. There was no evidence of alterations in renal function, so the significance of this finding is unclear.

21.2.2 Vaccination Schedules

Although it is not possible to give exact schedules for each of the veterinary vaccines available, certain principles are common to all methods of active immunization. Thus most vaccines require an initial series in which protective immunity is initiated, followed by revaccination (booster shots) at intervals to ensure that this protective immunity remains at an adequate level.

21.2.2.1 Initial Series

Because maternal antibodies passively protect newborn animals, it is not usually possible to successfully vaccinate animals very early in life. If stimulation of immunity is deemed necessary at this stage, the mother may be vaccinated during the later stages of pregnancy, the vaccinations being timed so that peak antibody levels are achieved at the time of colostrum formation. Once an animal is born, successful active immunization is effective only after passive immunity has waned. Since it is impossible to predict the exact time of loss of maternal immunity, the initial vaccination series will generally require administration of at least two and possibly more doses. Administration of vaccines to young animals is discussed in [Chapter 18](#).

The timing of initial vaccinations may also be determined by the disease. Some diseases are seasonal, and vaccines may be given before disease outbreaks are expected. Examples of these include the vaccine against the lungworm *Dictyocaulus viviparus* given in early summer just before the anticipated lungworm season, the vaccine against anthrax given in spring, and the vaccine against *Clostridium chauvoei* given to sheep before turning them out to pasture. Bluetongue of lambs is spread by midges (*Culicoides varipennis*) and is thus a disease of midsummer and early fall. Vaccination in spring will therefore protect lambs during the susceptible period.

21.2.2.2 Revaccination and Duration of Immunity

As pointed out in [Chapter 12](#), the phenomenon of immunological memory is not well understood; yet it is the persistence of memory cells, B cells, plasma cells, and T cells after vaccination that provides an animal with

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long-term protection. The presence of long-lived plasma cells is associated with persistent antibody production so that a vaccinated animal may have antibodies present in its bloodstream for many years after exposure to a vaccine. It is believed that these long-lived plasma cells are stimulated to survive by activation with nonspecific microbial molecules acting through toll-like receptors.

Revaccination schedules depend on the duration of effective protection ([Table 21-1](#)). This in turn depends on specific antigen content, whether the vaccine consists of living or dead organisms, and its route of

administration. In the past, relatively poor vaccines may have required frequent administration, perhaps as often as every 6 months, in order to maintain an acceptable level of immunity. Newer, modern vaccines usually produce a long-lasting protection, especially in companion animals; some may require revaccination only once every 2 or 3 years, whereas for others, immunity may persist for an animal's lifetime. Even killed viral vaccines may protect individual animals against disease for many years. Unfortunately, the minimal duration of immunity has, until recently, rarely been measured, and reliable figures are not available for many vaccines. Likewise, although serum antibodies can be monitored in vaccinated animals, tests have not been standardized and there is no consensus regarding the interpretation of these antibody titers. Even animals that lack detectable antibodies may well have significant resistance to disease. Nor is there much information available regarding long-term immunity on mucosal surfaces. In general, immunity against feline panleukopenia, canine distemper, canine parvovirus, and canine adenovirus is considered to be relatively long-lasting (longer than 5 years). On the other hand, immunity to feline rhinotracheitis, feline calicivirus, and chlamydiophilia is believed to be relatively short. One problem in making these statements is variability among individual animals and among different types of vaccine. Thus recombinant canine distemper vaccines may induce immunity of much shorter duration than conventional, modified live vaccines. There may be a great difference between the shortest and longest duration of immunological memory within a group of animals. Duration of immunity studies are confounded by the fact that in many cases older animals already show increased innate resistance. Different vaccines within a category may differ significantly in their composition, and although all vaccines may induce immunity in the short term, it cannot be assumed that all confer long-term immunity. Manufacturers use different master seeds and different methods of antigen preparation. The level of immunity required for most of these diseases is unknown. Likewise, a significant difference exists between the minimal level of immunity required to protect most animals and the level of immunity required to ensure protection of all animals.

Table 21-1 Estimated Minimum Duration of Immunity (DOI) of Select Commercially Available Canine Vaccine Antigens

Vaccine	Estimated Minimum DOI	Estimated Relative Efficacy (%)
Essential		
Canine distemper (MLV)	>7 years	>90
Canine distemper (R)	>3 years	>90
Canine parvovirus-2 (MLV)	>7 years	>90
Canine adenovirus-2 (MLV)	>7 years	>90
Rabies virus (K)	>3 years	>85
Optional		
Canine coronavirus (K or MLV)	N/A	N/A
Canine parainfluenza (MLV)	>3 years	>80
<i>Bordetella bronchiseptica</i> (ML)	<1 year	< 70
<i>Leptospira canicola</i> (K)	<1 year	< 50
<i>Leptospira grippotyphosa</i> (K)	<1 year	N/A
<i>Leptospira icterohaemorrhagiae</i> (K)	<1 year	< 75
<i>Leptospira pomona</i> (K)	<1 year	N/A
<i>Borrelia burgdorferi</i> (K)	1 year	< 75
<i>Borrelia burgdorferi</i> OspA (R)	1 year	< 75
<i>Giardia lamblia</i> (K)	<1 year	N/A
From Paul MA, Appel M, Barrett R, et al: <i>J Am Anim Hosp Assoc</i> 39:119-131, 2003.		
K, Killed; MLV, modified live virus; R, recombinant.		

Annual revaccination has been the rule for most animal vaccines since this approach is administratively simple and has the advantage of ensuring that an animal is regularly seen by a veterinarian. Recent information, however, indicates that some animal vaccines such as those against canine distemper or feline herpesvirus may induce protective immunity that can last for many years and that annual revaccination using these vaccines may be unnecessary. Unfortunately, there is insufficient information available on many vaccines to determine minimum vaccination intervals. A veterinarian should always assess the relative risks and benefits to an animal in determining the use of any vaccine and its frequency of administration. It may therefore be good practice to use serum antibody assays such as enzyme-linked immunosorbent assays, if available, to provide guidance on revaccination intervals. Persistent antibody titers may indicate protection, but this is not guaranteed, especially if cell-mediated immune mechanisms are important for protection. Likewise, animals with low or undetectable serum antibody levels may still be protected as a result of persistence of memory B and T cells capable of responding rapidly to reinfection.

Notwithstanding the discussion above, animal owners should be made aware that protection against an infectious disease can only be maintained reliably when vaccines are used in accordance with the protocol

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approved by the vaccine-licensing authorities. The duration of immunity claimed by a vaccine manufacturer is the minimum duration of immunity that is supported by the data available at the time the vaccine license is approved. This must always be taken into account when discussing revaccination protocols with an owner.

21.3 VACCINATION STRATEGIES

Although vaccination is a powerful tool for the control of infectious disease, its potential to prevent the spread of a disease or to eliminate a disease depends on selecting correct control strategies. If an infectious disease outbreak, such as one caused by foot-and-mouth virus, is to be rapidly controlled, it is vitally important to select the correct population to be vaccinated. The success of any mass vaccination program depends both on the proportion of animals vaccinated and on the efficacy of the vaccine. Neither of these factors will reach 100%, so it is essential to target the vaccine effectively. It is also the case that vaccines do not confer immediate protection, so the strategy employed will depend on the rate of spread of an infection. Vaccines may thus be given prophylactically, in advance of an outbreak, or reactively, in response to an existing outbreak. Both strategies have advantages and disadvantages. In general, prophylactic vaccination greatly reduces the potential for a major epidemic of a disease such as foot-and-mouth disease by reducing the size of a susceptible population. The effectiveness of this approach can be greatly enhanced by identifying high-risk individuals and ensuring that they are protected in advance of an outbreak.

It is generally not feasible to vaccinate an entire population of animals once a disease outbreak has occurred. However, two effective reactive vaccination strategies include “ring-vaccination,” which seeks to contain an outbreak by establishing a barrier of immune animals around an infected area, and “predictive-vaccination,” which seeks to vaccinate the animals on farms likely to contribute most to the future spread of disease. Reactive vaccination in this way can ensure that an epidemic is not unduly prolonged. A prolonged “tail” to an epidemic commonly results from the disease “jumping” to a new area. Well-considered, predictive vaccination may well prevent these jumps. Thus a combination of prophylactic and reactive vaccination will likely yield the most effective results.

21.4 VACCINE ASSESSMENT

To assess the efficacy of a vaccine, animals must first be vaccinated and then challenged. The percentage of vaccinated animals that survive this challenge can then be measured. It is important, however, to determine the percentage of nonvaccinated control animals that also survive the challenge. The true efficacy of a vaccine, called the preventable fraction (PF), is calculated as follows:

$$PF = \frac{(\% \text{ of controls dying} - \% \text{ of vaccinates dying})}{\% \text{ of controls dying}}$$

For example, a challenge that kills 80% of controls and 40% of vaccinates shows that the PF of the vaccine is as follows:

$$PF = \frac{80 - 40}{80} = 50 \%$$

Good, effective vaccines should have a PF of at least 80%. Obviously, less effective vaccines are acceptable if safe and if nothing better is available.

21.5 FAILURES IN VACCINATION

There are many reasons why a vaccine may fail to confer protective immunity on an animal (Figure 21-1).

21.5.1 Incorrect Administration

In many cases vaccine failure is due to unsatisfactory administration. For example, a live vaccine may have died as a result of poor storage, the use of antibiotics in conjunction with live bacterial vaccines, the use of chemicals to sterilize the syringe, or the excessive use of alcohol when swabbing the skin. Sometimes, animals given vaccines by nonconventional routes may not be protected. When large flocks of poultry or mink are to be vaccinated, it is common to administer the vaccine either as an aerosol or in drinking water. If the aerosol

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FIGURE 21-1 A simple classification of the ways in which a vaccine may fail to protect an animal.

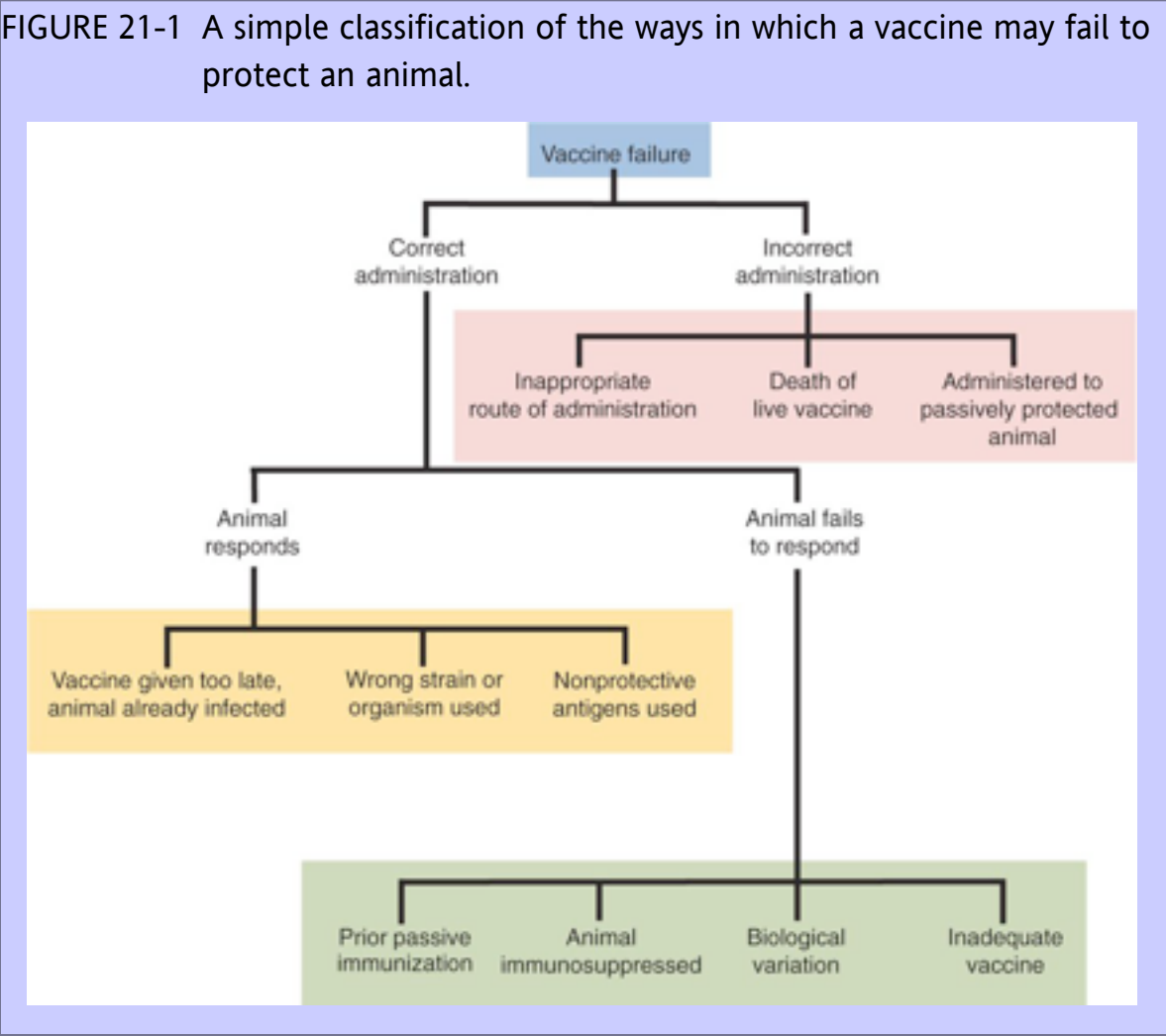
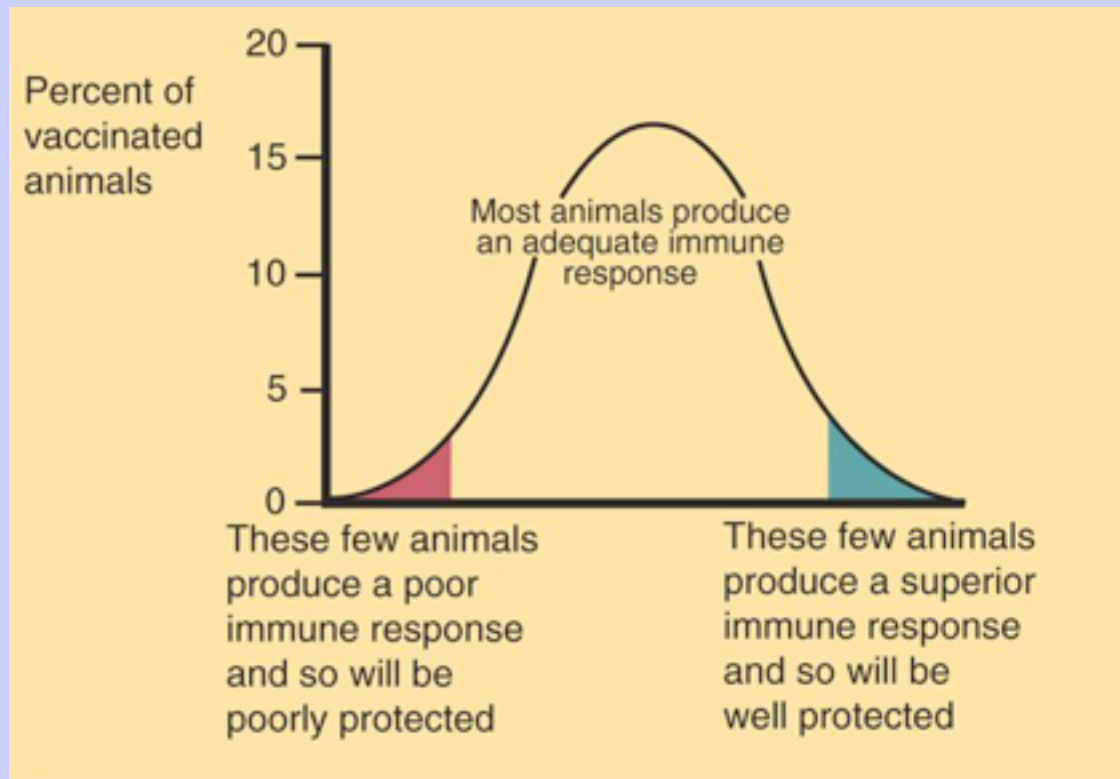


FIGURE 21-2 The normal distribution of protective immune responses in a population of vaccinated animals. No vaccine can be expected to protect 100% of a population.



is not evenly distributed throughout a building, or if some animals do not drink, they may receive insufficient vaccine. Animals that subsequently develop disease may be interpreted as cases of vaccine failure.

21.5.2 Failure to Respond

Occasionally, a vaccine may actually be ineffective. The method of production may have destroyed the protective epitopes, or there may simply be insufficient antigen in the vaccine. Problems of this type are uncommon and can generally be avoided by using only vaccines from reputable manufacturers.

More commonly, an animal may simply fail to mount an immune response. The immune response, being a biological process, never confers absolute protection and is never equal in all members of a vaccinated population. Since the immune response is influenced by a large number of genetic and environmental factors, the range of immune responses in a large random population of animals tends to follow a normal distribution. This means that most animals respond to antigens by mounting an average immune response, whereas a few will mount an excellent response and a small proportion will mount a poor immune response ([Figure 21-2](#)).

This group of poor responders may not be protected against infection in spite of having received an effective vaccine. Therefore it is essentially impossible to protect 100% of a random population of animals by vaccination. The size of this unreactive portion of the population will vary between vaccines, and its significance will depend

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on the nature of the disease. Thus, for highly infectious diseases against which herd immunity is poor and in which infection is rapidly and efficiently transmitted, such as foot-and-mouth disease, the presence of unprotected animals could permit the spread of disease and would thus disrupt control programs. Likewise, problems can arise if the unprotected animals are individually important; for example, companion animals. In contrast, for diseases that are inefficiently spread, such as rabies, 70% protection may be sufficient to effectively block disease transmission within a population and may therefore be quite satisfactory from a community health viewpoint.

Another type of vaccine failure occurs when the normal immune response is suppressed. For example, heavily parasitized or malnourished animals may be immunosuppressed and should not be vaccinated. Some virus infections induce profound immunosuppression. Animals with a major illness or high fever should normally not be vaccinated unless for a compelling reason. Stress may reduce a normal immune response, probably because of increased steroid production; examples of such stress include pregnancy, fatigue, malnutrition, and extremes of cold and heat. This type of immunosuppression is discussed in detail in [Chapter 35](#). The most important cause of vaccine failure of this type is passively derived maternal immunity in young animals, as described in [Chapter 18](#).

21.5.3 Correct Administration and Response

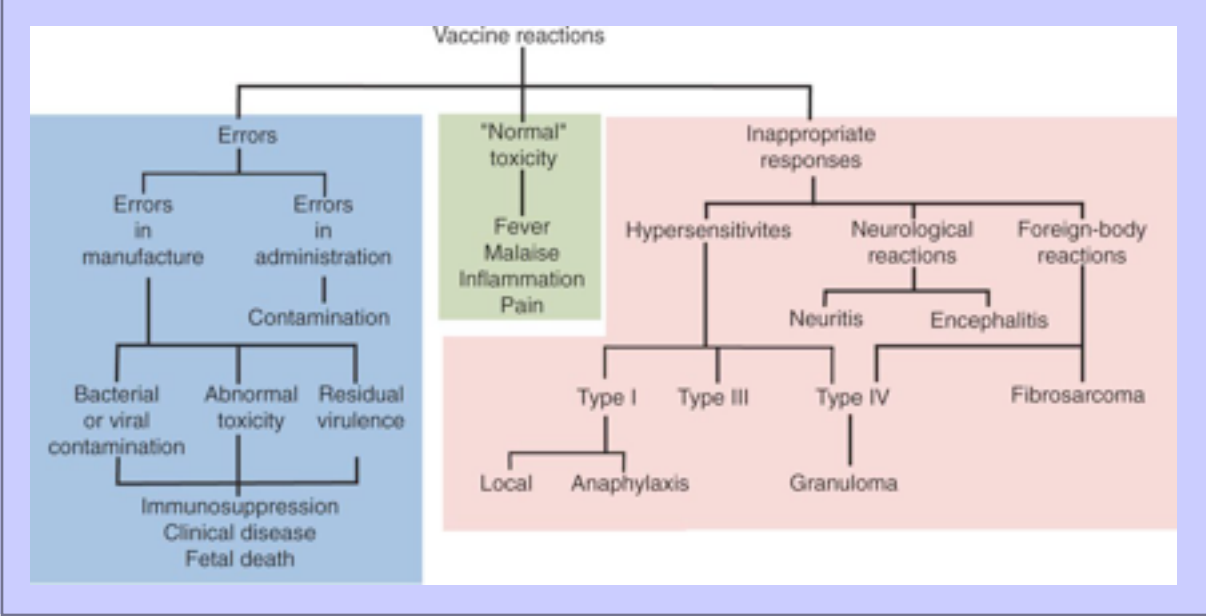
Even animals given an adequate dose of an effective vaccine may fail to be protected. If the vaccinated animal was incubating the disease before inoculation, the vaccine may be given too late to affect the course of the disease. Alternatively, the vaccine may contain the wrong strain of organisms or the wrong (nonprotective) antigens.

21.6 ADVERSE CONSEQUENCES OF VACCINATION

Vaccination continues to be the only safe, reliable, and effective way of protecting animals against the major infectious diseases. Vaccine-related toxicity is usually rare, mild, and transient, and hypothetical side effects must not dominate our perceptions. Nevertheless, the use of vaccines is not free of risk. Residual virulence and toxicity, allergic responses, disease in immuno-deficient hosts, neurological complications, and harmful effects on the fetus are the most significant risks associated with the use of vaccines ([Figure 21-3](#)). Veterinarians should use only licensed vaccines, and the manufacturer's recommendations should be carefully followed. Before using a vaccine, the veterinarian should consider the likelihood that an adverse event will happen, as well as the possible consequences or severity of this event. These factors must be weighed against the benefits to the animal. Thus a common but mild complication may require a different consideration than a rare, severe complication.

The issue of the risk associated with vaccination remains, in large part, a philosophical one, since the advantages of vaccination are well documented and extensive whereas the risk for adverse effects is poorly documented and, in many cases, largely hypothetical. Nevertheless, established facts should be recognized, unsubstantiated allegations rebutted by sound data, and uncertainties acknowledged. For example, there is absolutely no evidence that vaccination itself

FIGURE 21-3 A simple classification of the major adverse effects of vaccination.



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leads to ill health. While difficult to prove a negative, competent statistical analysis has consistently failed to demonstrate any general adverse effect of vaccination.

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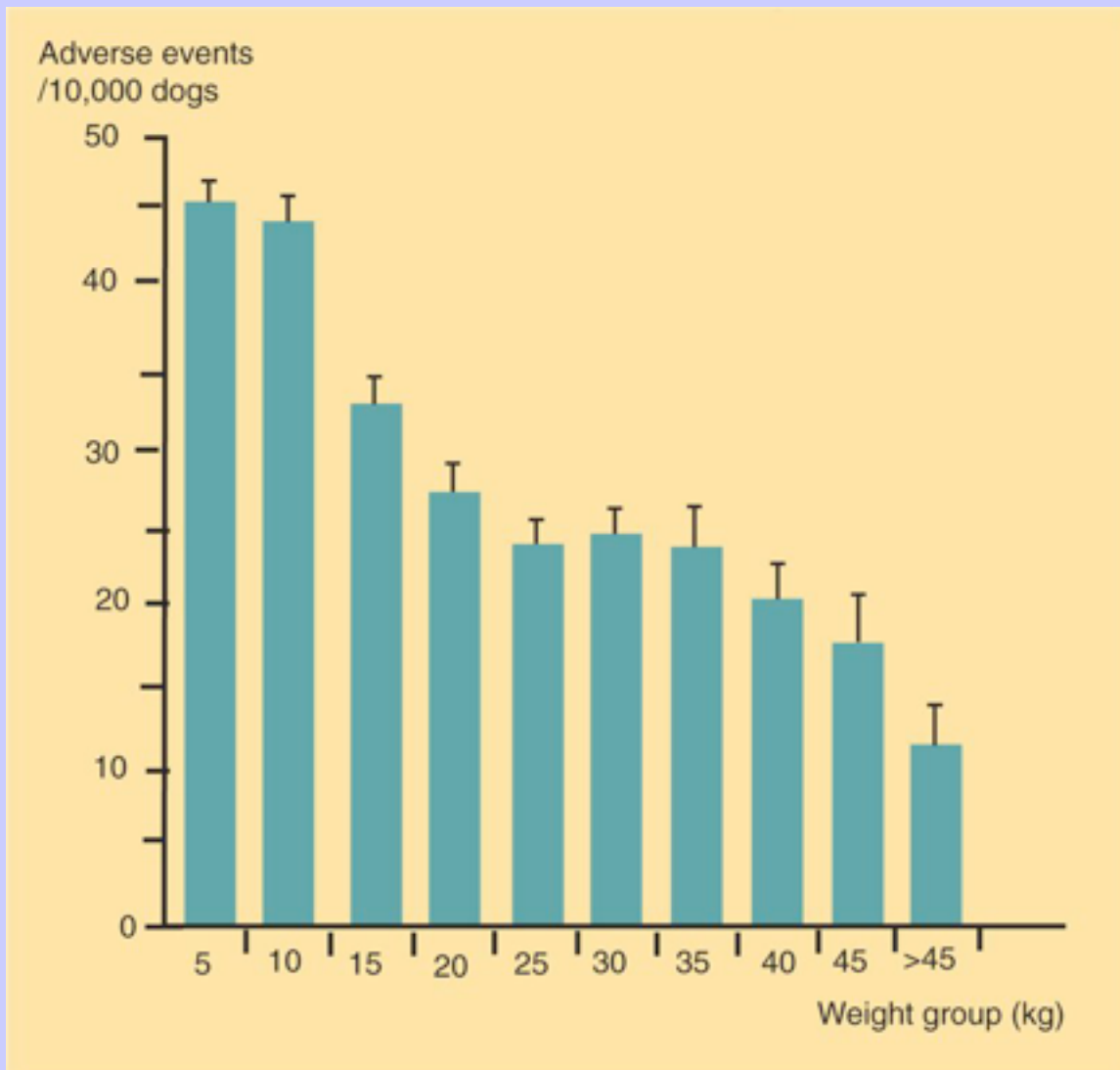
Traditionally, adverse events resulting from vaccine administration are reported voluntarily by veterinarians to manufacturers or government agencies. The resulting figures have been impossible to analyze satisfactorily for two major reasons. First, reporting is voluntary, so significant underreporting occurs. Many adverse events are regarded as insignificant, or it may be inconvenient for a veterinarian to report them. Second, very little data have been available on the number of animals vaccinated. While manufacturers know the number of doses of vaccine sold, they are unable to measure the number of animals vaccinated. It is therefore a significant event when it becomes possible to determine the prevalence of vaccine-associated adverse events (VAAEs) occurring in over a million dogs. This has proved possible by examining the electronic records of a very large general practice. The use of a standardized reporting system and the large population studied permits infrequent adverse events to be recorded with very much greater accuracy than previously possible. In a groundbreaking study by Dr. Larry Glickman and his colleagues, the prevalence of adverse events that occurred within three days of vaccine administration was determined. Out of 1,226,159 dogs vaccinated, there were 4678 adverse events recorded (38.2/10,000 dogs). Of these events 72.8% occurred on the same day the vaccine was administered, 31.7% were considered to be allergic reactions, and 65.8% were considered “vaccine reactions” and were likely due to toxicity. Additional analysis of these events indicated that the risk of adverse events was significantly greater for small than for large dogs (Figure 21-4); for neutered than for sexually intact dogs; and for dogs that received multiple vaccine doses. Each additional vaccine dose administered increased the risk of an adverse event occurring by 27% in small dogs (less than 10 Kg) and by 12% in dogs heavier than 12 Kg. High-risk breeds included Dachshunds, Pugs, Boston Terriers, Miniature Pinschers, and Chihuahuas. Overall, the increased incidence of adverse events in small dogs and its relationship to multiple dosing suggests that we should look carefully at the practice of giving the same dose of vaccine to all dogs irrespective of their size.

A similar study examined the incidence of VAAEs following the administration of 1,258,712 doses of vaccine to 496,189 cats. Adverse events numbering 2560 were reported (51.6/10,000 cats vaccinated). The risk was greatest

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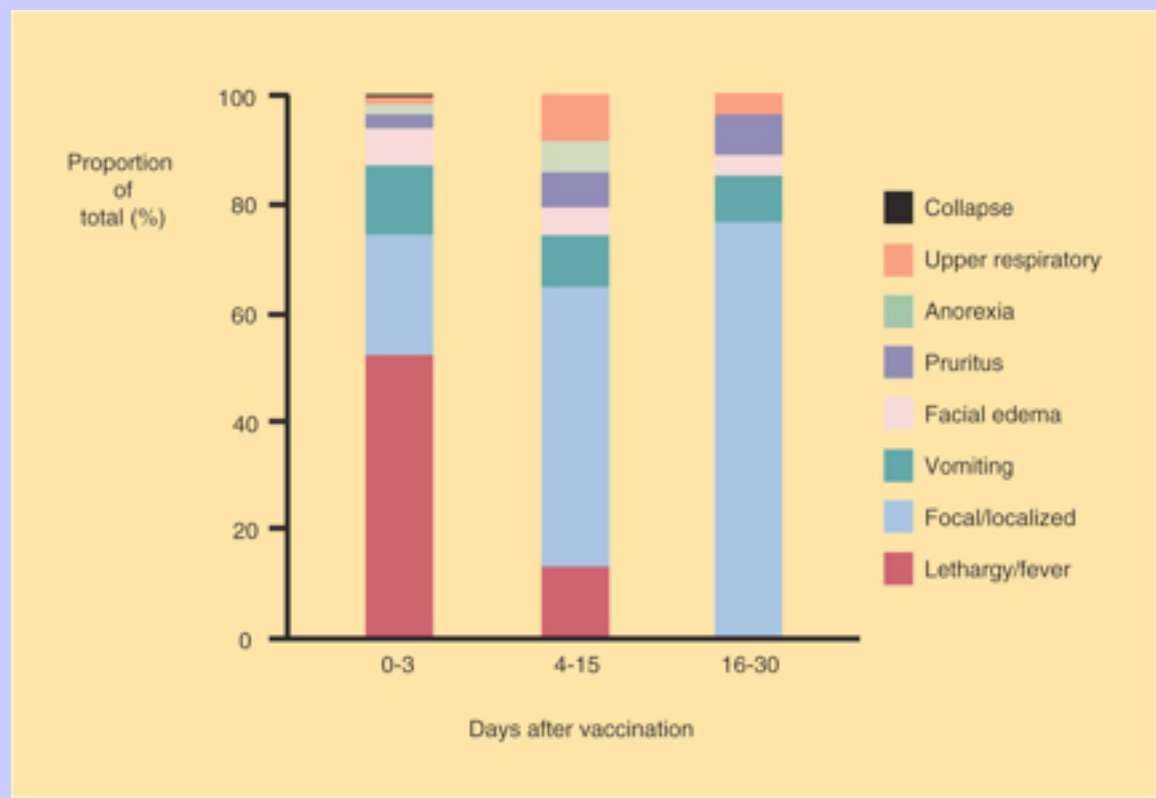
for cats 1 year old. For unknown reasons, risk was greater in neutered than in sexually intact cats. Lethargy was the most commonly reported event ([Figure 21-5](#)). The number of adverse events

FIGURE 21-4 Vaccine-associated adverse events (VAAEs) are much more likely to occur in small rather than in large dogs. Mean \pm SEM VAAE rates by 5-kg weight groups in 1,226,159 dogs vaccinated at 360 veterinary hospitals from January 1, 2002, to December 31, 2003. These adverse events were diagnosed within 3 days of vaccine administration. (From Moore GE et al: *JAVMA* 227:1102-1108, 2005.)



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FIGURE 21-5 Distribution of types of vaccine-associated adverse events diagnosed during various periods after vaccination in 496,189 cats administered one or more vaccines from January 1, 2002, to December 31, 2004. (From Moore GE et al: *JAVMA* 231: 94-100, 2007.)



increased significantly when multiple vaccinations were given during an office visit.

It should be pointed out that identification of an adverse event is based on the clinical judgment of the attending veterinarian and is itself subject to bias. Standard case definitions of VAAEs are not yet available. On the other hand, the importance of such bias is reduced by the use of such a large database.

21.6.1 “Normal” Toxicity

Vaccines commonly elicit transient inflammatory reactions, and some degree of inflammation is required for the efficient induction of protective immune responses. This may cause pain. Thus the sting produced by some vaccines may present problems not only to the animal being vaccinated but also, if the animal reacts violently, to the vaccinator. More commonly, local swellings may develop at the reaction site. These may be firm or edematous and may be warm to the touch. They appear about one day after vaccination and can last for about a week. Unless an injection-site abscess develops, these swellings leave little trace. Vaccines containing killed Gram-negative organisms may be intrinsically toxic owing to the presence of endotoxins that can cause cytokine

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release, leading to shock, fever, and leukopenia. Although such a reaction is usually only a temporary inconvenience to male animals, it may be sufficient to provoke abortion in pregnant females. Thus it may be prudent to avoid vaccinating pregnant animals unless the risks of not giving the vaccine are considered to be too great.

21.6.2 Inappropriate Responses

Vaccines may cause rare but serious allergic reactions. For example, type I hypersensitivity can occur when an animal produces IgE in response not only to the immunizing antigen but also to other antigens found in vaccines, such as egg antigens or antigens from tissue culture cells. All forms of hypersensitivity are more commonly associated with multiple injections of antigens and therefore tend to be associated with the use of killed vaccines. It is important to emphasize that a type I hypersensitivity is an immediate response to an antigen and occurs within a few minutes or hours after exposure to an antigen. Reactions occurring more than 2 or 3 hours after administration of a vaccine are likely not type I hypersensitivity reactions.

Type III hypersensitivity reactions are also potential hazards. These may cause intense local inflammation, or they may present as a generalized vascular disturbance such as purpura. A type III reaction can occur in the eyes of dogs vaccinated against infectious canine hepatitis (see [Chapter 27](#)). Some rabies vaccines may induce a local complement-mediated vasculitis leading to ischemic dermatitis and local alopecia. This type of reaction is most often seen in small dogs such as Dachshunds, Miniature Poodles, Bichon Frises, and Terriers.

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Type IV hypersensitivity reactions may occur in response to vaccination, but a more common reaction is granuloma formation at the site of inoculation. This may be a response to depot adjuvants containing alum or oil. Vaccines containing a water-in-oil adjuvant produce larger and more persistent lesions at injection sites than vaccines containing alum and aluminum hydroxide. These lesions can be granulomas or sterile abscesses. If the skin is dirty at the injection site, these abscesses may become infected.

Under some circumstances vaccination may trigger autoimmunity. For example, rabies vaccines that contain central nervous tissue may provoke auto-immune encephalitis. A polyneuritis (Gullain-Barré syndrome) has been associated with the use of cer-tain virus vaccines (most notably swine influenza) in humans, and at least one case has been reported in a dog following vaccination with a polyvalent distemper-hepatitis-parvovirus vaccine (see [Chapter 32](#)). The pathogenesis of this syndrome is unclear.

Postvaccinal canine distemper virus encephalitis is a rare complication that may develop after administration of a modified live canine distemper vaccine. The affected animal may show aggression, incoordination, and seizures or other neurological signs. The pathogenesis of this condition is unknown, but it may be due to residual virulence, increased susceptibility, or triggering of a latent paramyxovirus by the vaccine.

21.6.3 Errors in Manufacture or Administration

Some problems associated with vaccine use may be due to poor production or administration. Thus some modified live vaccines may retain the ability to cause disease. For example, some modified live herpes vaccines or calicivirus vaccines given intranasally may spread to the oropharynx and result in persistent infection. Indeed, such a virus vaccine may infect (and protect) other animals in contact. Even if these vaccines do not cause overt disease, they may reduce the rate of growth of farm animals with significant economic consequences.

Some vaccines may trigger a mild immunosuppression. For example, some modified live parvovirus vaccines may cause a transient decrease in lymphocyte blastogenesis responses or even a lymphopenia in some puppies.

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Not all strains of canine parvovirus-2 are immunosuppressive. Some polyvalent canine viral vaccines can cause a transient drop in absolute lymphocyte numbers and their responses to mitogens (see [Chapter 35](#), [Figure 35-2](#)). This occurs even though the individual components of these vaccines may not have this effect. Several vaccine combinations may cause these changes between 5 and 11 days after vaccination. Thus, for example, a combination of canine adenovirus type 1 or type 2 with canine distemper virus is especially suppressive of canine lymphocyte responses to mitogens. This suppression of T cell responses may be accompanied by simultaneous enhancement of B cell responses and raised immunoglobulin levels. Thus rather than being a pure immunosuppressive effect, it may simply reflect a transient change in the Th1/Th2 balance.

Vaccines such as bluetongue vaccine have been reported to cause congenital anomalies in the offspring of ewes vaccinated while pregnant. The stress from vaccination may also be sufficient to reactivate latent infections: for example, activation of equine herpesvirus has been demonstrated following vaccination against African horse sickness. Mucosal disease may develop in calves vaccinated against BVD (see [Chapter 18](#)).

21.6.4 Injection Site–Associated Sarcomas

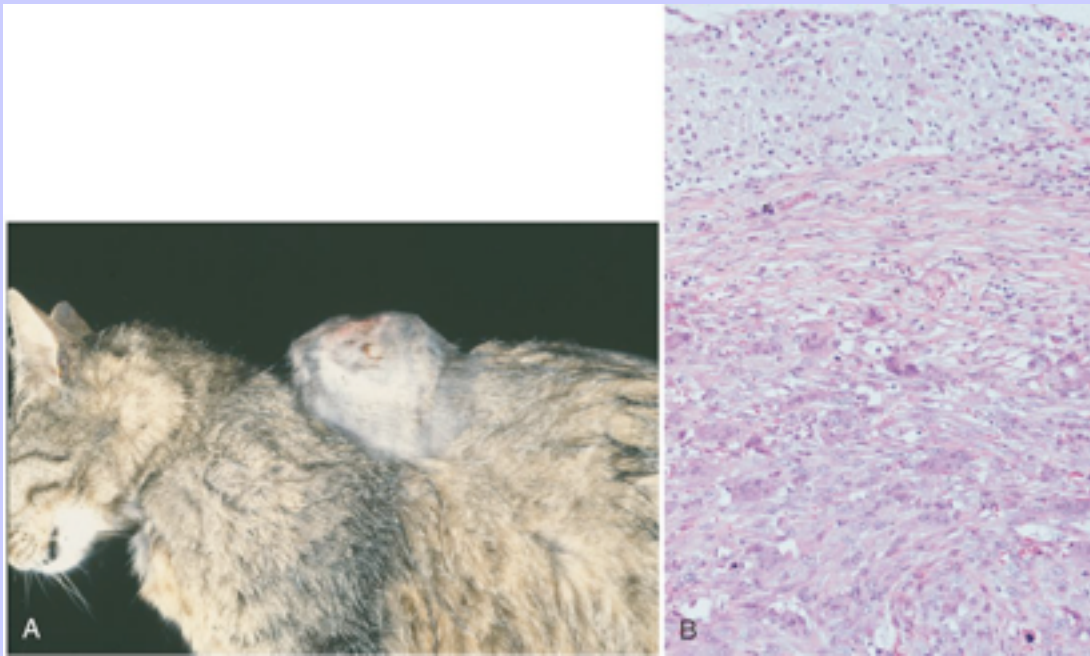
Most local reactions to vaccines in cats resolve rapidly. In some cats, however, tumors have developed at these injection sites many months after injection. They have typically been found in the cervical/interscapular and femoral regions, sites at which vaccines are commonly injected ([Figure 21-6](#)). Injection site–associated sarcoma cells have large irregular nuclei, often pleomorphic with a high mitotic index. There may be a central area of necrosis. Aggregates of lymphocytes and macrophages may surround the tumor. These macrophages may have foamy cytoplasm containing bluish granular material. The tumors are mainly fibrosarcomas, malignant histiocytomas, and osteosarcomas. Less common forms include rhabdomyosarcomas, hemangiosarcomas, chondrosarcomas, liposarcomas, and lymphosarcomas.

21.6.4.1 Epidemiology

Epidemiological studies have linked the development of these sarcomas to vaccination. Thus the first appearance of these tumors coincided with the introduction of new, potent, inactivated, adjuvanted vaccines such as those directed against rabies and feline leukemia. Cats with sarcomas occurring at sites where vaccines are currently administered were compared with cats that developed sarcomas at non–vaccine injection sites. Cats receiving feline leukemia virus (FeLV) vaccine were 5.5 times more likely to develop a sarcoma at the injection site than cats that had not received a vaccine. There was a twofold increase in

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FIGURE 21-6 **A**, A postvaccinal sarcoma in a cat. Note its characteristic position over the scapulae, where the vaccine has been administered subcutaneously. **B**, A histological section of a postvaccinal sarcoma. This is a fibrosarcoma showing long interwoven bundles of spindle cells (H&E stain). (Courtesy Dr. M.J. Hendrick.)



risk with rabies vaccination. However, the risk was not enormously high. It was calculated that 1 to 3.6 sarcomas developed per 10,000 FeLV and rabies vaccines administered. The risk did increase with the number of doses of vaccine administered—a 50% increase following one dose, a 127% increase following two doses, and a 175% increase following three or four vaccines given simultaneously. Vaccine-associated sarcomas tend to occur in younger animals and tend to be larger and more aggressive than sarcomas arising at other sites. They metastasize in 25% to 70% of cases. In one study, injection site sarcomas developed on average 26 months after the last rabies vaccination given and 11 months after FeLV vaccination. Global, Web-based surveys suggest a somewhat lower prevalence of sarcomas (0.63 sarcomas/10,000 cats or 0.32 sarcomas/10,000 doses of all vaccines, or one sarcoma from 31,000 doses administered). It must be pointed out, therefore, that the risks for developing a sarcoma are considerably smaller than the disease risks incurred by unvaccinated cats. Similar vaccination-related injection site sarcomas have been reported in ferrets.

21.6.4.2

Possible Mechanisms

The pathogenesis of these sarcomas is unclear, but it is assumed that carcinogenesis occurs through multiple steps associated with prolonged inflammation. The potent adjuvants found in modern vaccines will result in immune responses and hence protection lasting for several years. In addition, these products are administered by the convenient subcutaneous route. As a result, an irritating adjuvant may persist at the injection site for a

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long time. Nevertheless, tumor development has also been associated with the use of nonadjuvanted vaccines and even with injection of substances other than vaccines, as well as the presence of persistent sutures or implanted microchips. There is no evidence that feline sarcoma virus, feline immunodeficiency virus, or FeLV causes these tumors.

Prolonged irritation will increase the activation state of the cells involved in inflammation and tissue repair. The repair process involves the production of stem cells that can differentiate to replace damaged ones. These stem cells are long lived and so have plenty of opportunities to accumulate mutations. Signaling pathways may be activated in stem cells, and these pathways will promote cellular self-renewal. Chronic, prolonged irritation could lead to an increase in local stem cells and the possibility that some will mutate.

During chronic inflammation, macrophages secrete growth factors and angiogenic factors that enhance cell growth. These factors will upregulate nuclear factor kappa-B (NF- κ B) activity in affected tissues. Oxidants released from activated macrophages may act as carcinogens, especially in cells that are dividing rapidly.

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Although the mechanisms are unclear, NF- κ B promotes both malignant transformation and metastases and may promote cancer cell formation by inhibiting apoptosis of premalignant cells.

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Fibroblasts are stimulated to proliferate at sites of chronic inflammation and wound healing. In some of these fibroblasts the *sis* oncogene is activated, whereas in others, there appear to be mutations in the gene coding for the tumor suppressor factor. The *sis* oncogene codes for the platelet-derived growth factor (PDGF) receptor, and vaccine-associated sarcomas have been shown to express both PDGF and its receptor. In contrast, non-vaccine-associated tumors and normal cat lymphocytes are PDGF-negative. It has been suggested, therefore, that lymphocytes within the vaccine-associated sarcomas secrete PDGF, which then serves as a growth factor for the fibroblasts. This combination of abnormalities could result in the loss of growth control in the fibroblasts engaged in the chronic inflammatory process.

The tumor suppressor gene p53 codes for a nuclear protein that regulates the cell cycle. Wild-type p53 increases in response to cell damage. This prevents the cell from moving through the cell cycle and permits DNA repair before the cell divides. If the cell is severely damaged, p53 triggers apoptosis and so prevents cell damage being transmitted to the next generation. In cells where p53 is absent or mutated, damaged cells can continue to divide, giving rise to abnormal and possibly malignant cells. Evidence suggests that as many as 60% of vaccine-associated sarcomas may express mutated p53. Cats with p53 in their cytoplasm showed a shorter time for tumor recurrence than cats with cells in which p53 was restricted to their nucleus.

Interleukin-23 (IL-23) is a proinflammatory cytokine produced by activated dendritic cells and phagocytic cells. IL-23 acts on T cells, promoting inflammatory responses and upregulating some inflammatory metalloproteases and stimulating angiogenesis. However it also reduces CD8 T cell infiltration. By reducing T cell infiltration, IL-23 permits the growth of cancer cells. Animals deficient in IL-23 show increased resistance to chemical carcinogenesis, and transplanted tumors are growth-restricted in these animals. Is it possible that this cytokine is produced in large amounts in chronic inflammatory lesions and so promotes local tumor growth.

Notwithstanding the above, there is no evidence to prove that injecting less-inflammatory products can reduce the incidence of sarcomas. No specific brands of vaccine, no specific manufacturers, and no other vaccination-associated factors have been associated with an increased prevalence of sarcomas.

To minimize the risks of tumors developing at vaccination sites, it is recommended that when multiple vaccines are given, they be administered at standardized sites on the animal and away from sites where tumor management is difficult. For example, current recommendations are to inject rabies vaccine on the caudal half

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of the right side and FeLV vaccine on the caudal half of the left side of the cat's body ("rabies, right; leukemia, left"). If possible, the site of vaccine administration and the product used should be recorded for each vaccine to help in assessing risk factors. Nonadjuvanted vaccines appear to induce less severe irritation and present a correspondingly lower risk for tumor formation. Successful treatment requires a combination of aggressive surgical excision and adjunctive therapy including radiation, immunotherapy (such as IL-2 treatment), and chemotherapy.

21.6.5 Vaccine-Associated Autoimmune Disease

It is widely believed that the prevalence of autoimmune disease in domestic pets, especially dogs, has risen in recent years. Some investigators have attributed this rise to excessive use of potent vaccines. This link is by no means proven; nevertheless, there is limited evidence that supports an association between vaccination and autoimmunity. A retrospective analysis of the history of dogs presenting with immune-mediated hemolytic anemia (IMHA) (see [Chapter 32](#)) showed that 15 of 70 dogs with IMHA had been vaccinated within the previous month as compared with a randomly selected control group in which none had been vaccinated. Dogs with IMHA that developed within a month of vaccination differed in some clinical features from dogs with IMHA unassociated with prior vaccination. Epidemiological studies using very large databases tend to confirm this effect, in that they show an approximately threefold increase in diagnoses of autoimmune thrombocytopenia, and twofold increase in diagnoses of IMHA in dogs in the 30 days following vaccination, compared to other time periods. The overall incidence of these diseases, however, is low, and they can be diagnosed at times not temporally associated with vaccination. Vaccination may therefore serve as a stimulus for these diseases in some dogs, but other, undefined, stimuli must also exist.

Contaminating thyroglobulin found in some vaccines (usually from the presence of fetal bovine serum) can lead to the production of antithyroid antibodies in vaccinated dogs. Lymphocytic thyroiditis has been found in 40% of Beagle dogs on necropsy, but there was no association detected between vaccination and the development of this thyroiditis.

It is well recognized that Guillain-Barré syndrome, an autoimmune neurological disease of humans, can be triggered by administration of some vaccines such as influenza vaccine. Likewise, in animals, the administration of potent, adjuvanted vaccines stimulates the transient production of a variety of autoantibodies. Vaccines containing potent adjuvants may trigger the development of low levels of autoantibodies to connective tissue components such as fibronectin and laminin.

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21.6.6 Vaccine-Induced Osteodystrophy

Vaccination of some Weimaraner puppies with a modified live virus (MLV) vaccine may lead to the development of a severe hypertrophic osteodystrophy. The disease appears within 10 days of administration of a vaccine containing modified live canine distemper vaccine. Systemic signs include anorexia, depression, and fever; gastrointestinal, nervous, and respiratory symptoms; and symmetrical metaphyseal lesions with painful swollen metaphyses. Radiological examination shows radiolucent zones in the metaphyses, flared diaphyses, and formation of new periosteal bone. Hind and fore limbs are equally affected. It is possible that the condition is triggered in genetically susceptible animals by modified live canine distemper virus. The disease responds well to corticosteroid therapy. In many cases, these dogs show a preexisting immune dysfunction with low concentrations of one or more immunoglobulin classes, recurrent infections, and inflammatory disease (see [Chapter 34](#)). It has been suggested that Weimaraners are especially susceptible to this condition and that they receive only killed virus vaccines.

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A mild transient polyarthritis has been reported to occur in other dogs following vaccination. Dogs show a sudden onset of lameness with swollen and painful joints within two weeks of vaccination. The dogs recover within two days. No specific breed or vaccine has been associated with this problem. Vaccination against calicivirus has been associated with polyarthritis and a postvaccination limping syndrome in cats.

21.7 ADVERSE EFFECT PRINCIPLES

In determining whether a vaccine causes an adverse effect, the following principles should apply.

1. Consistency—the clinical responses should be the same if the vaccine is given to a different group of animals, by different investigators, and irrespective of the method of investigation.
2. Specificity—the association should be distinctive and the adverse event linked specifically to the vaccine concerned. An adverse event may be caused by vaccine adjuvants and additives other than the active component.
3. Temporal relation—administration of the vaccine should precede the earliest manifestations of the event or a clear exacerbation of a continuing condition.

21.8 PRODUCTION, PRESENTATION, AND CONTROL OF VACCINES

The production of veterinary vaccines is controlled by the Animal and Plant Health Inspection Service of the U.S. Department of Agriculture in the United States, by the Health of Animals Branch of the Canada Department of Agriculture in Canada, and by the Veterinary Medicines Directorate in the United Kingdom. In general, regulatory authorities have the right to license establishments where vaccines are produced and to inspect these premises to ensure that the facilities are appropriate and that the methods employed are satisfactory. All vaccines must be checked for safety and potency. Safety tests include confirmation of the identity of the organism used and of the freedom of the vaccine from extraneous organisms (i.e., purity), as well as tests for toxicity and sterility. Because the living organisms or antigens found in vaccines normally die or degrade over a period of time, it is necessary to ensure that they will be effective even after storage. It is usual therefore to use an antigen in generous excess of the dose required to protect animals under laboratory conditions, and potency is tested both before and after accelerated aging. Vaccines that contain killed organisms, although much more stable than living ones, also contain an excess of antigens for the same reason. Vaccines approved for licensing on the basis of challenge exposure studies must usually show evidence of protection in 80% of vaccinated animals; at the same time, at least 80% of the unvaccinated controls must develop evidence of disease after challenge exposure (the 80 : 80 efficacy guideline). The route and dose of administration indicated on the vaccine label should be scrupulously heeded since these were probably the only route and dose tested for safety and efficacy during the licensing process. Vaccines usually have a designated shelf life, and although properly stored vaccines may still be potent after the expiration of this shelf life, this should never be assumed. Correct storage and handling are essential. All expired vaccines should be discarded. Adverse reactions should always be reported to the appropriate licensing authorities as well as to the vaccine manufacturer.

Because modified live vaccines carry with them the risks for residual virulence and for contamination with other agents, certain countries will not approve their use.

Inactivated vaccines are commonly available in liquid form and usually contain suspended adjuvant. These should not be frozen, and they should be shaken well before use. The presence of preservatives such as phenol or

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merthiolate will not control massive bacterial contamination, and multidose containers should be discarded after partial use. Many vaccines containing MLVs are susceptible to heat inactivation but are much more resistant if lyophilized. Remember, however, that intense sunlight and heat can destroy even lyophilized vaccines. They store well but should be kept cool and away from light and should only be reconstituted with the fluid provided by the manufacturer.

21.9 SOME ANTIBACTERIAL VACCINES

21.9.1 Toxoids

The immunoprophylaxis of tetanus is restricted to toxin neutralization. Tetanus toxoid in an aluminum hydroxide suspension is given for routine prophylaxis, and a single injection will induce protective immunity in 10 to 14 days. Conventional immunological wisdom would suggest that the previous use of immune globulin should interfere with the immune response to toxoid and must therefore be avoided. This is not a problem in practice, however, and both may be successfully administered simultaneously (at different sites) without problems. This may be because of the relatively small amount of immune globulin usually needed to protect animals.

Some veterinary vaccines combine both toxoid and killed bacteria in a single dose by the simple expedient of formolizing a whole culture. These products, sometimes called anacultures, are used to vaccinate against *Clostridium haemolyticum* and *Clostridium perfringens*. Trypsinization of the anaculture may make it more immunogenic. Toxoids, usually incorporated with an alum adjuvant, are available for most clostridial diseases and for infections caused by toxigenic staphylococci.

21.9.2 Bacterins

Vaccines containing killed bacteria are called bacterins. It is usual to kill the bacteria with formaldehyde and to incorporate them with alum or aluminum hydroxide adjuvants. As with other dead vaccines, the immunity produced by bacterins is relatively short-lived, usually lasting for not longer than 1 year and sometimes for a considerably shorter period. For instance, formolized swine erysipelas (*E. rhusiopathiae*) vaccine protects for only 4 to 5 months, and *S. equi* bacterins give immunity for less than 1 year, even though recovery from a natural case of strangles may confer a lifelong immunity in horses.

Bacterins may be improved by adding purified immunogenic antigens to the killed bacteria. Thus *Escherichia coli* bacterins against enteric colibacillosis may be enriched and made much more effective by the addition of K88 or K99 pilus antigens. Antibodies to these antigens block binding of *E. coli* to the intestinal wall and thus contribute significantly to protection. Similarly, *Mannheimia* bacterins enriched in the leukotoxoid show improved efficacy over conventional bacterins. Purified bacterial components such as the surface antigens of *M. haemolytica* may also be effective vaccine components.

One problem encountered, especially when using coliform and *Campylobacter* vaccines, is strain specificity. Several different antigenic types of each organism commonly occur, and successful vaccination requires immunization with appropriate bacterial strains. This is sometimes not possible if a commercial vaccine must be employed. One method of overcoming this difficulty is to use autogenous vaccines. These are vaccines that contain organisms obtained either from infected animals on the farm where the disease problem is occurring or from the infected animal itself. These can be very successful if carefully prepared, since the vaccine will contain all the antigens required for protection in that particular location. As an alternative to the use of autogenous vaccines, some manufacturers produce polyvalent vaccines containing a mixture of antigenic types. For example,

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leptospirosis vaccines commonly contain up to five different serovars. This practice, although effective, is inefficient, since only a few of the antigenic types employed may be appropriate in any given situation.

A major advance in the development of vaccines against Gram-negative bacteria is the use of common core antigens. As pointed out in [Chapter 2](#), the outer layer of the Gram-negative bacterial cell wall consists of lipopolysaccharide. This lipopolysaccharide consists of a variable oligosaccharide (O antigen) bound to a highly conserved core polysaccharide and lipid A. The O antigen varies greatly among Gram-negative bacteria so that an immune response against one O antigen confers no immunity against bacteria bearing other O antigens. In contrast, the underlying core polysaccharide is similar between Gram-negative bacteria of different species and genera. Thus an immune response directed against this common core structure has the potential to protect against a wide variety of different Gram-negative bacteria.

Mutant strains of *E. coli* (J5) and *Salmonella enterica minnesota* or *Salmonella enterica typhimurium* (Re) have been used as sources of core antigen. J5 is a rough mutant that is deficient in uridine diphosphate galactose 4-epimerase. As a result the organism makes an incomplete oligosaccharide side chain, having lost most of the outer lipopolysaccharide structure (see [Chapter 2](#), [Figure 2-2](#)). Immunization with J5 thus provides protection against *E. coli*, *Klebsiella pneumoniae*, *Actinobacillus pleuropneumoniae*, *Haemophilus influenzae* (type B), and *S. enterica typhimurium*. J5 has been reported to protect calves against organisms such as *S. enterica typhimurium* and *E. coli* and pigs against *A. pleuropneumoniae*. The most encouraging results have been obtained in protection against coliform mastitis.

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21.9.3 Living Bacterial Vaccines

Successful living bacterial vaccines include strains 19 and RB51 of *Brucella abortus*. Another successful living vaccine is that employed for the prevention of anthrax. Older anthrax vaccines used Pasteur's technique of culturing the bacteria at a relatively high temperature (42° to 43°C) so that their virulence is reduced. The anthrax vaccines currently available for animals contain capsuleless mutants that remain capable of forming spores. The vaccine is prepared as a spore suspension and is administered with saponin.

A rough strain of *Salmonella enterica dublin* (strain 51) is used in Europe to give good protection to calves when administered at 2 to 4 weeks of age. As discussed earlier, immunity to salmonellosis involves macrophage activation and is thus relatively nonspecific. For this reason, strain 51 may also give good protection against *S. enterica typhimurium*.

21.10 SOME ANTIVIRAL VACCINES

Because of the lack of antiviral drugs, vaccination is the only effective method for the control of most virus diseases in domestic animals. As a result, the development of viral vaccines is, in many ways, more advanced than the development of their bacterial counterparts. It has, for example, proved relatively easy to attenuate many viruses so that effective vaccines containing MLV derived from tissue culture are readily available.

As discussed in [Chapter 20](#), MLV vaccines are usually good immunogens, but their use may involve certain risks. The most important problem encountered is residual virulence. One serious example of this was the development of clinical rabies in some dogs and cats following administration of older strains of MLV rabies vaccine. Some strains of infectious bovine rhinotracheitis and equine herpesvirus-1 vaccines may cause abortion when given to pregnant cows or mares, respectively, and MLV bluetongue vaccines may cause disease in fetal lambs if given to pregnant ewes (see [Chapter 18](#)). More commonly, the residual virulence in these vaccines causes a mild disease. Thus intraocular or intranasal rhinotracheitis or calicivirus vaccines may cause a transient conjunctivitis or rhinitis in

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cats. MLV infectious bursal disease vaccines, some canine parvovirus-2 vaccines, and some BVD vaccines can cause a mild immunosuppression.

Transient side effects such as these, which may otherwise be regarded as inconsequential, can be of major significance in the broiler chicken industry, where even a minor slowing in growth can have major economic results. Thus two strains of infectious bronchitis vaccine are available. The Massachusetts strain is mildly pathogenic but a good immunogen, whereas the Connecticut strain is nonpathogenic but a poor immunogen. To minimize complications it is common to use the Connecticut strain for primary vaccination and, if boosters are required, to use the Massachusetts strain subsequently. Similarly, of the two major vaccine strains of Newcastle disease, the LaSota strain is a good immunogen but may provoke mild adverse reactions. In contrast, the B1 strain is considerably milder but is less immunogenic, especially if given in drinking water. In other situations, birds may be primed with the very mild G2 Newcastle disease strain. Then, in the face of severe challenge, they may be boosted with a relatively virulent live vaccine.

Because of problems of this nature, persistent attempts have been made to minimize residual virulence in vaccines. One method involves the use of temperature-sensitive (ts) mutants. Ts strains of bovine herpesvirus-1, for example, will grow only at temperatures a few degrees lower than normal body temperature. When this organism is administered intranasally, it is able to colonize the relatively cool nasal mucosa but is unable to invade the rest of the body. Thus the vaccine can stimulate local immunity without incurring the risk for a systemic invasion. (It also has the advantage that its activity is not blocked by maternal immunity.) Some vaccine viruses may persist in vaccinated animals and cause a prolonged carrier state. Although this is a problem largely associated with herpesviruses, concerns have been expressed that the widespread use of MLV vaccines may serve to seed viruses into animal populations and that untoward consequences may develop in the future. This is a threat not to be taken lightly.

An alternative approach to overcoming the problems caused by modified live vaccines involves the increasing use of inactivated and subunit vaccines. Excellent inactivated vaccines are available against diseases such as foot-and-mouth disease, equine herpesvirus-4 (rhinopneumonitis), pseudorabies, feline panleukopenia, feline herpes (rhinotracheitis), and rabies. A genetically engineered subunit vaccine directed against the gp70 envelope antigen of FeLV is also available. At their best, these vaccines confer immunity comparable in strength and duration to that induced by MLV vaccines, with the assurance that they are free of residual virulence.

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22 CHAPTER 22 Acquired Immunity to Bacteria and Fungi

22.1 KEY POINTS

- Antibodies neutralize bacterial toxins.
- Antibodies alone will opsonize bacteria. Antibodies and complement may opsonize bacteria or kill them directly through the membrane attack complex.
- T cell-mediated activation of macrophages is required to kill intracellular bacteria.
- Under some circumstances, especially in mycobacterial disease, an inappropriate Th2 response rather than the required Th1 response may lead to severe disease and death.
- Bacteria may resist destruction by multiple mechanisms.
- Cell-mediated immune responses are usually required to protect against fungal infections.

Although animals live in environments densely populated with bacteria, the vast majority of these organisms neither invade animal tissues nor cause disease. This is unsurprising for several reasons. First, the combined efforts of the innate and acquired immune systems are sufficient to prevent invasion. Second, even organisms that successfully invade the animal body gain very little by harming their host. On the contrary, illness or death of the host animal may well reduce the survival of the bacteria and is therefore normally avoided. Indeed, many bacteria are essential for the animal's well-being, since they maintain an environment on body surfaces that is hostile to other potential invaders. They also assist in the digestion of foods such as celluloses and promote the normal development of the immune system. Nevertheless, many commensal bacteria are also potential pathogens. For example, *Clostridium tetani* and *Clostridium perfringens* are commonly found among the intestinal flora of horses, and *Bordetella bronchiseptica* is found in the nasopharynx of healthy swine. Bacterial disease is not, therefore, an inevitable consequence of the presence of pathogenic organisms on body surfaces. The development of disease is related to many other factors, including the response of the host, the presence of damaged tissues, the location of the bacteria within the body, and the disease-producing power (or virulence) of the bacteria. Only when the balance between host immunity and bacterial virulence is upset will disease or death result.

Antimicrobial immunity consists of an early innate response followed by a more sustained adaptive response. Recognition of invading bacteria through toll-like receptors (TLRs) and other receptors induces inflammation, cytokine release, and complement activation. If this is insufficient to eliminate the invaders, acquired immune mechanisms take over. Thus dendritic cells and macrophages ingest invading bacteria and initiate acquired immunity by secreting cytokines and triggering both T and B cell responses. The importance of these innate defenses is emphasized by the observation that the resistance of chickens to *Salmonella enterica* serotype *typhimurium* appears to be linked to allelic variations in TLR4 while the resistance of foals to *Rhodococcus equi* depends on TLR2.

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TLRs are responsible in large part for the initial recognition of invading bacteria. Binding of microbial products to TLRs triggers a signal cascade that activates genes that are vitally important in host defense. Some pathogenic bacteria can interfere with the TLR-signaling pathways and use them to escape immune destruction. Thus they may use the TLR-mediated pathways to induce interleukin-10 (IL-10) release or block TLR-mediated signaling. For example, products from *Candida spp.*, *Yersinia spp.*, or *Mycobacteria spp.* can trigger signaling through TLR2 leading to production of IL-10 by regulatory T cells and inhibition of interferon- γ (IFN- γ) production. Phospholipids from *Treponema spp.* can block cell activation induced by TLR3, TLR4, and TLR9. Organisms such as *Leptospira*

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spp. have lipopolysaccharides that are recognized by TLR2 but not by TLR4. Likewise flagellin from *Helicobacter pylori* is not properly recognized by TLR5.

22.2 ACQUIRED IMMUNITY

There are five basic mechanisms by which the acquired immune responses combat bacterial infections ([Figure 22-1](#)). These are (1) the neutralization of toxins or enzymes by antibody, (2) the killing of bacteria by antibodies and complement, (3) the opsonization of bacteria by antibodies and complement, resulting in their phagocytosis and destruction, (4) the destruction of intracellular bacteria by activated macrophages, and (5) direct killing of bacteria by cytotoxic T cells and natural killer (NK) cells. The relative importance of each of these processes depends on the species of bacteria involved and on the mechanisms by which they cause disease.

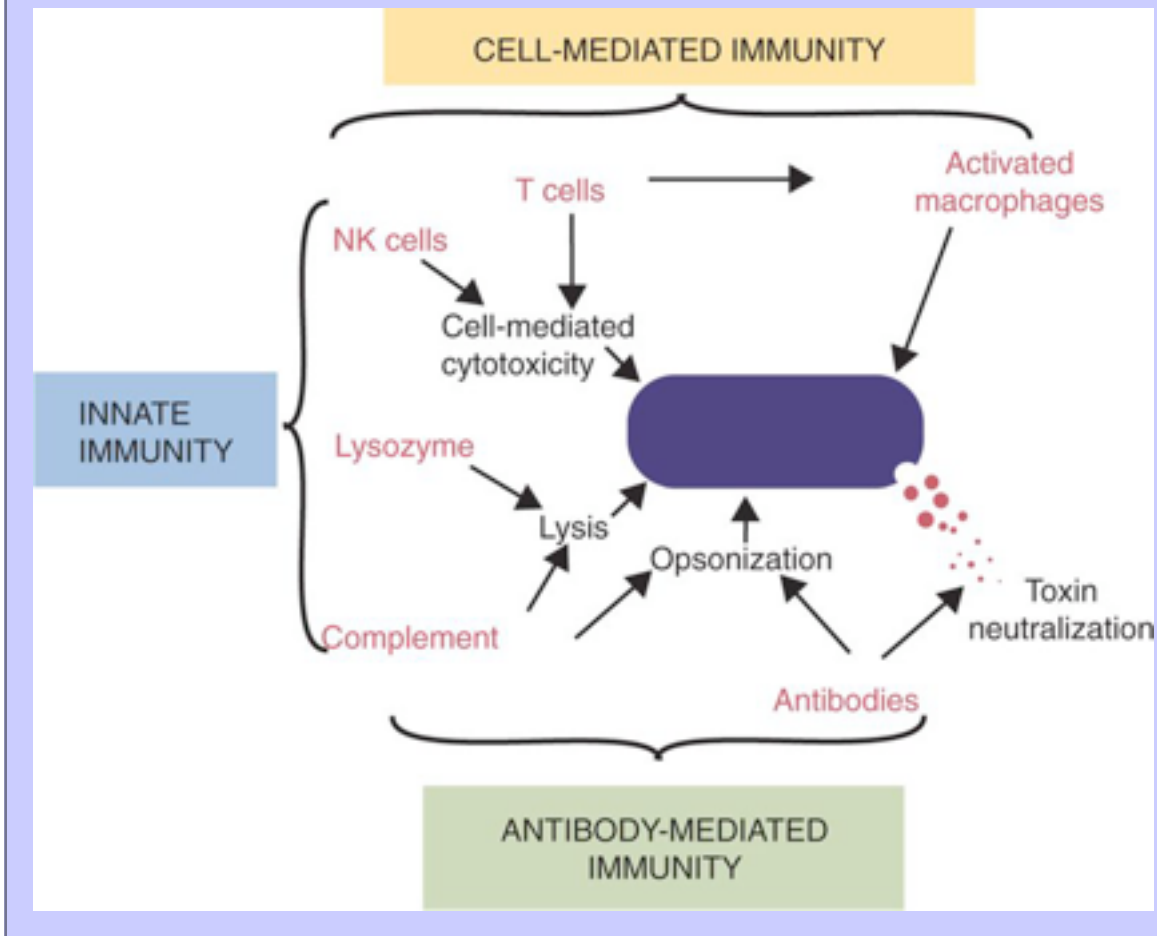
22.2.1 Immunity to Toxigenic Bacteria

In disease caused by toxigenic bacteria such as *Clostridia* spp. or *Bacillus anthracis*, the immune response must not only eliminate the invading bacteria but also neutralize their toxins. Destruction of the bacteria may, however, be difficult if they are embedded in a mass of necrotic tissue, and toxin neutralization is a priority. Neutralization occurs when antibody prevents the toxin from binding to its receptors on a target cell. The neutralization process therefore involves competition between receptors and antibodies for the toxin molecule. Once the toxin has combined with its receptors, antibodies are relatively ineffective in reversing this combination.

22.2.2 Immunity to Invasive Bacteria

Protection against invasive bacteria is usually mediated by antibodies directed against the surface antigens of the bacteria. Efficient phagocytosis requires that the surface of bacteria be coated with a layer of opsonin that will be recognized by phagocytic cells. These opsonins are mainly either antibodies or C3b in addition to the innate opsonins such as mannose-binding lectin. Activation of complement by bacteria

FIGURE 22-1 The mechanisms by which the immune responses can protect the body against bacterial invasion.



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through the alternative and lectin pathways leads to the binding of C3b to their surface. Antibodies not only act as effective opsonins in their own right but also increase the binding of C3b by activating the classical complement pathway. Antibody directed against capsular (K) antigens may neutralize the antiphagocytic properties of the capsule, thus opsonizing the bacteria and permitting destruction by phagocytic cells to take place. In bacteria lacking capsules, antibodies directed against O antigens act as opsonins. A more subtle protective effect occurs when antibodies are produced against strains of *Escherichia coli* carrying the pilus antigens F4 (K88) or F5 (K99). In this case, the antibodies interfere with the expression of the pilus antigens, and it has been claimed that they are eventually able to cause deletion of the genetic material (plasmid) that codes for these antigens. Once the adherence pili are deleted, these strains of *E. coli* cannot bind to the intestinal wall and thus are no longer pathogenic.

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The importance of bacterial capsules in immunity is seen in anthrax. *B. anthracis* possesses both a capsule and an exotoxin. Antitoxic immunity is protective but slow to develop. In addition, toxin production tends to be prolonged, since the organism is encapsulated and phagocytic cells have difficulty eliminating it. As a result, death is usually inevitable in unvaccinated animals. The vaccine commonly employed against animal anthrax contains an unencapsulated but toxigenic strain of *B. anthracis*. Given in the form of spores that can germinate,

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the unencapsulated bacteria are eliminated by phagocytic cells before dangerous amounts of toxin are synthesized but not before antitoxic immunity is simulated.

While some bacteria are destroyed by phagocytosis, others are killed when free in the circulation. In unsensitized animals, the bacteria are destroyed by complement acting through the alternate or lectin pathways—innate defense mechanisms. Lacking sialic acid, bacterial cell walls inactivate factor H and thus permit the production of the alternate C3 convertase (C3bBbP). As a result, the bacteria are either opsonized or lysed. The importance of this pathway is seen in bovine mycoplasma infections, in which pathogenic and nonpathogenic organisms may be differentiated on the basis of their ability to activate the alternate pathway. Nonpathogenic mycoplasmas activate the pathway; pathogenic ones do not.

Activation of the terminal complement components leads to the development of membrane attack complexes (MAC). These MACs may be unable to insert themselves into the complex carbohydrates of the microbial cell wall. However, lysozyme in the blood may digest the cell wall and enable the MACs to gain access to the lipid bilayer of the inner bacterial membrane.

Molecule for molecule, immunoglobulin M (IgM) is about 500 to 1000 times more efficient than IgG in opsonization and about 100 times more potent than IgG in sensitizing bacteria for complement-mediated lysis. During a primary immune response, therefore, the quantitative deficiency of the IgM response is compensated for by its quality, thus ensuring early and efficient protection.

The traditional view of antibodies was that they alone could not kill microorganisms: They simply marked microorganisms for destruction. That view is now known to be incorrect. There are many examples of antibodies that have direct antimicrobial activities. Thus antibodies against *E. coli* may be bacteriostatic since they interfere with the secretion of the iron chelator enterochelin and so prevent bacterial iron scavenging. IgM and IgG antibodies against *Borrelia burgdorferi* damage surface proteins on the bacteria and so are bactericidal in the absence of complement. There is also evidence that antibodies are able to generate oxidants and so kill bacteria directly.

22.2.2.1

The Heat-Shock Protein Response

Many new proteins are induced in cells by stresses such as a raised temperature, starvation, and exposure to oxygen radicals, toxins such as heavy metals, protein synthesis inhibitors, or viral infections. The heat-shock proteins (HSPs) are the best understood of these new proteins. HSPs are present in all organisms at very low levels at normal temperatures. Mild stress such as a low-grade fever will induce HSP production and increase their levels significantly. For example, HSP levels climb from 1.5% to 15% of the total protein in stressed *E. coli*. There are three major bacterial HSPs: HSP 90, HSP 70, and HSP 60. (The number refers to their molecular weight.) When a bacterium is phagocytosed and exposed to the respiratory burst within a neutrophil, the stress results in the production of bacterial HSP. Thus HSP 60 is the dominant antigen in infections caused by mycobacteria, *Coxiella burnetii*, *Legionella*, *Treponema* spp., and *Borrelia* spp. These HSPs are highly antigenic for several reasons. First, they are produced in abundance in the infected host; second, they are readily processed by antigen-presenting cells; third, the immune system may possess unusually large numbers of cells capable of responding to HSPs. Some γ/δ T cells may preferentially recognize bacterial HSPs. An anti-HSP response may therefore be a major defense against many bacterial pathogens.

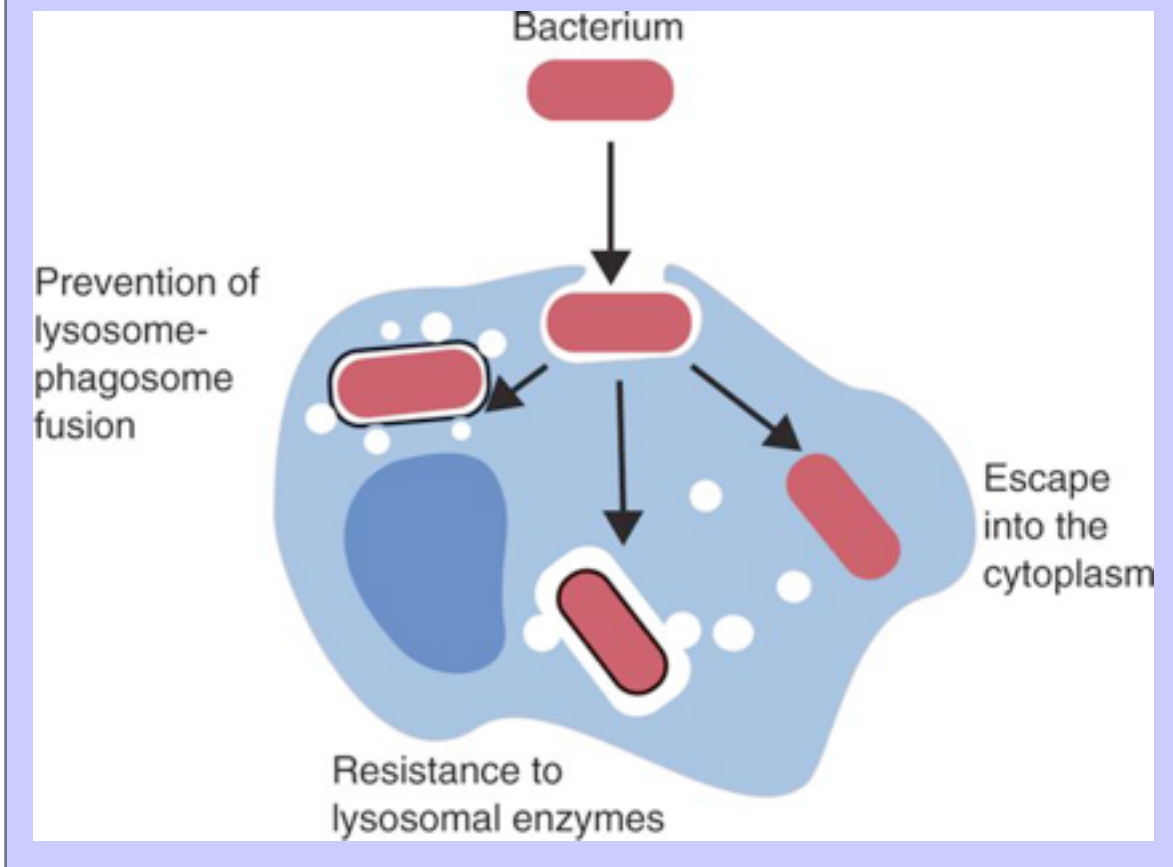
22.2.3 Immunity to Intracellular Bacteria

As discussed in [Chapter 16](#), some bacteria such as *Brucella abortus*, *Mycobacterium tuberculosis*, *Campylo*

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FIGURE 22-2 The mechanisms by which intracellular bacteria can evade intracellular destruction.



bacter jejuni, *R. equi*, *Listeria monocytogenes*, *Corynebacterium pseudotuberculosis*, *C. burnetii*, and some serotypes of *S. enterica* can readily grow inside macrophages. In addition, *L. monocytogenes* can travel from cell to cell without exposure to the extracellular fluid. Bacteria employ many different strategies to ensure their survival within macrophages ([Figure 22-2](#)). Some bacteria employ a resistant coat to protect themselves against lysosomal enzymes ([Table 22-1](#)). For example, the cell wall waxes of *C. pseudotuberculosis* make that organism resistant to lysosomal enzymes. Other bacteria ensure that they are never exposed to these enzymes by interfering with phagosomal maturation. *Salmonella* serotype typhimurium prevents assembly of the NADPH oxidase (NOX) complex. *Mycobacteria*, *Aspergillus flavus*, *B. abortus*, and *Chlamydomphila psittaci* can establish themselves within vacuoles that exclude proteases and oxidants by blocking lysosome-phagosome fusion. In the case of *M. tuberculosis*, the bacterium enters the macrophage via cholesterol-enriched membrane microdomains that are coated on the cytoplasmic side with a protein (tryptophan-aspartate-containing coat protein, or TACO) that prevents phagosome maturation. Thus lysosomes cannot fuse with the phagosome. They remain distributed within the cytoplasm, and the bacteria continue to survive and grow. *Mycobacteria* can also prevent acidification of phagosomes by preventing fusion of the proton pump ATPase with the vacuolar membrane so that lysosomal

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cathepsins remain inactive. A third mechanism used by bacteria to avoid destruction is simply to escape from the phagosome and persist free within the cytoplasm surrounded by a coat of polymerized actin. This method is employed by some mycobacteria and by *L. monocytogenes*.

Table 22-1 Facultative Intracellular Bacteria and Their Mechanisms of Survival

Organism	Method of Intracellular Survival
<i>Brucella abortus</i>	Resistant cell wall
	Prevents phagosome maturation
<i>Corynebacterium pseudotuberculosis</i>	Resistant cell wall
<i>Listeria monocytogenes</i>	Neutralizes respiratory burst
	Escapes into the cytosol
<i>Mycobacterium tuberculosis</i>	Lipid cell wall
	Prevents phagosome maturation
	Suppresses antigen presentation
	Detoxifies oxidants
<i>Salmonella enterica</i>	Prevents phagosome maturation
	Modifies endosomal trafficking
	Detoxifies oxidants
	Downregulates NOS2 and NOX
<i>Rhodococcus equi</i>	Survives in phagosomes

Many bacteria manipulate the host's cytokine responses to their own advantage. For example, IFN- γ and tumor necrosis factor- α (TNF- α) produced by primed T cells can generate M1 macrophages, acidify the phagosomes, and so kill mycobacteria. Responding mycobacteria may suppress the T cell responses by inducing synthesis of IL-6, IL-10, and transforming growth factor- β to prolong their own survival. IL-10 is especially effective in inhibiting macrophage activation, suppressing oxidant production, and downregulating antigen processing by reducing major histocompatibility complex (MHC) class II expression.

Protection against intracellular bacteria is mediated by M1 cells. Although macrophages from unimmunized animals are normally incapable of destroying these bacteria, this ability is acquired about 10 days after onset of infection when the macrophages are activated (see [Chapter 16](#)). The response of these activated macrophages tends to be nonspecific, particularly in *Listeria* infections, and activated macrophages are able to destroy many normally resistant bacteria. IFN- γ , especially in association with TNF- α , greatly enhances the production of NO and NO $_2^-$. Thus an animal recovering from an infection with *L. monocytogenes* develops increased resistance to infection by *M. tuberculosis*. The development of these activated macrophages often coincides with the appearance of delayed (type IV) hypersensitivity responses to intradermally administered antigen (see [Chapter 28](#)).

Both CD4⁺ and CD8⁺ cells are also involved in immunity to *Listeria* spp. CD8⁺ cytotoxic T cells lyse *Listeria*- or mycobacteria-infected cells and so complement the Th1 cells that activate the macrophages. *R. equi*-infected macrophages are recognized and killed by CD8⁺ T cells in an MHC class I unrestricted manner. 289 290

It has been observed that protective immunity against intracellular bacteria cannot be induced by vaccines containing killed bacteria. Only vaccines containing living bacteria are protective. This difference is probably due to the differential stimulation of helper T cell populations by live and dead bacteria. Thus infection of mice with live *B. abortus* stimulates Th1 cells to secrete IFN- γ . Immunization of these mice with *Brucella* protein extracts induces Th2 cells to secrete IL-4. Live but not dead *L. monocytogenes* or *B. abortus* organisms induce macrophage secretion of TNF- α . Conversely, killed *Brucella* organisms stimulate IL-1 production to a greater extent than live bacteria. Resistance to these intracellular bacteria is generally short-lived, persisting for only as long as viable bacteria remain in the body. (Tuberculosis is an exception to this, in which case memory is prolonged.)

If, in a bacterial disease, it is observed that dead vaccines do not give good protection, that serum cannot confer protection, that antibody levels do not relate to resistance, and that delayed hypersensitivity reactions can be elicited to the bacterial antigens, then the possibility that cell-mediated immunity may play an important role in resistance to the causative organism should be considered and the use of vaccines containing living bacteria should be contemplated.

22.2.4 Modification of Bacterial Disease by Immune Responses

An animal's immune response will clearly influence the course and severity of an infection. At best, it will result in a cure. In the absence of a cure, however, the infection may be profoundly modified. This also depends on whether a cell-mediated or antibody response is generated. Thus the type of helper T cells induced by an infection may affect the course of disease. As described in [Chapter 16](#), cell-mediated responses are usually required to control intracellular bacteria. Only activated macrophages can prevent the growth of these bacteria. Macrophage activation requires that Th1 cells produce IFN- γ . When the macrophages are activated, these M1 cells can localize or cure these infections. If, in contrast, the immune response against these bacteria inappropriately stimulates Th2 responses, cell-mediated immunity fails to develop, M2 macrophages are generated, and chronic progressive disease results. This is readily seen in mycobacterial diseases. For example, in humans, leprosy occurs in two distinct forms called tuberculoid and lepromatous leprosy. Tuberculoid leprosy is characterized by an intense cell-mediated immune response with macrophage activation and minimal antibody responses to the leprosy bacillus. Their response is dominated by Th1 cells and M1 macrophages. Lesions in this form of the disease contain very few organisms. Lepromatous leprosy, in contrast, is characterized by very high antibody levels and poor cell-mediated responses. Humans with lepromatous leprosy mount an immune response, employing Th2 cells that secrete IL-4 and IL-10. The IL-10 reduces the production of IL-12, which in turn decreases IFN- γ secretion by Th1 cells and generates M2 macrophages. This reduces the patients' ability to control *Mycobacterium leprae* and their lesions contain enormous numbers of bacteria. The prognosis of lepromatous leprosy is much poorer than for tuberculoid leprosy.

A similar diversity of lesions is seen in Johne's disease of sheep. Some animals develop a lepromatous form of the disease, in which their intestinal lesions contain enormous numbers of bacteria ([Figure 22-3](#)) and little histological evidence of a cell-mediated response. In contrast, other sheep may develop a tuberculoid form of the disease, in which the lesions

FIGURE 22-3 The two forms of Johne's disease in sheep. **A**, Section of terminal ileum from a case of lepromatous Johne's disease, showing abundant acid-fast organisms within large infiltrating macrophages. **B**, Section of terminal ileum from a case of tuberculoid Johne's disease, showing very few acid-fast bacteria and a significant lymphocyte infiltration. Ziehl-Nielsen stain. (Courtesy Dr. C.J. Clarke.)

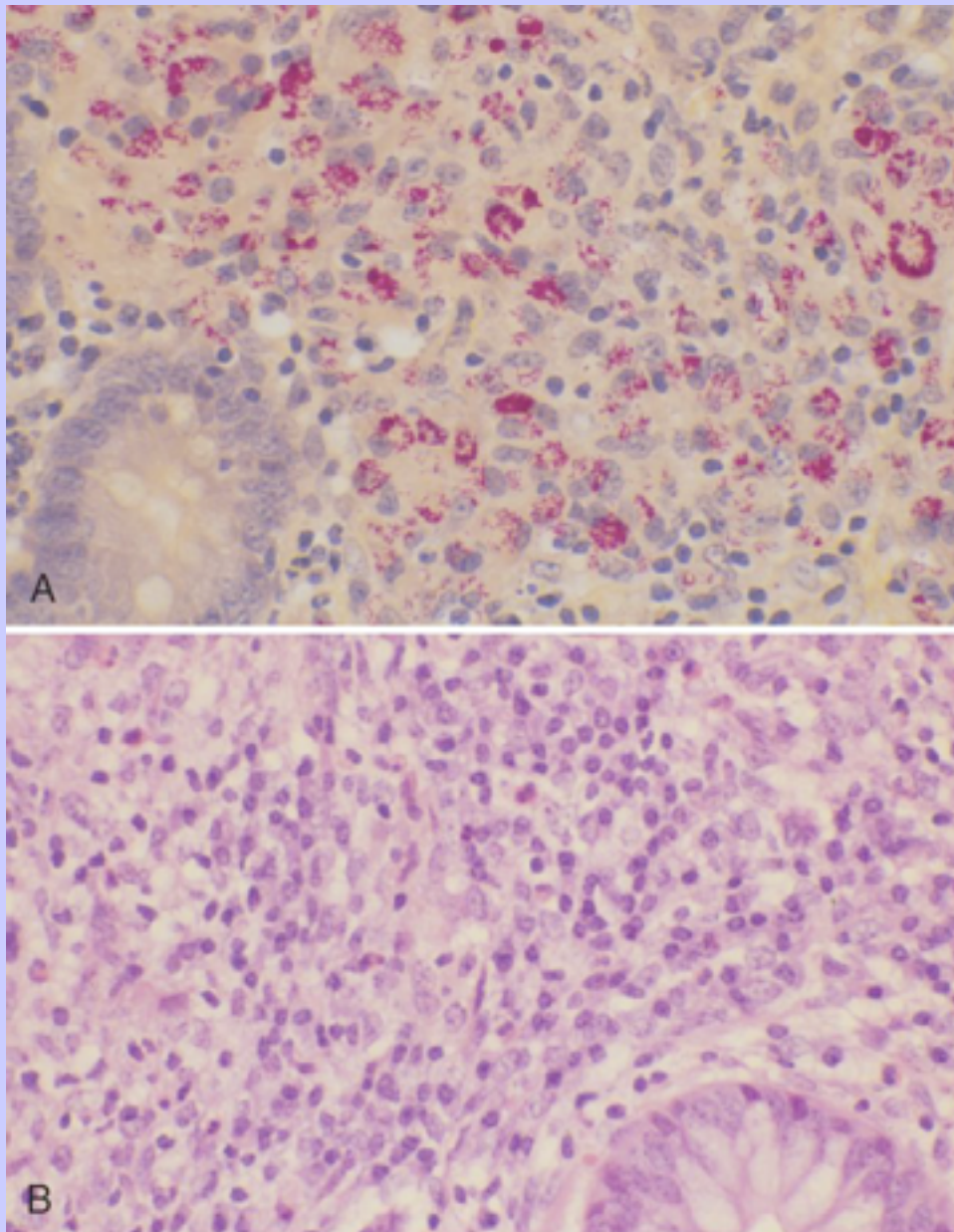
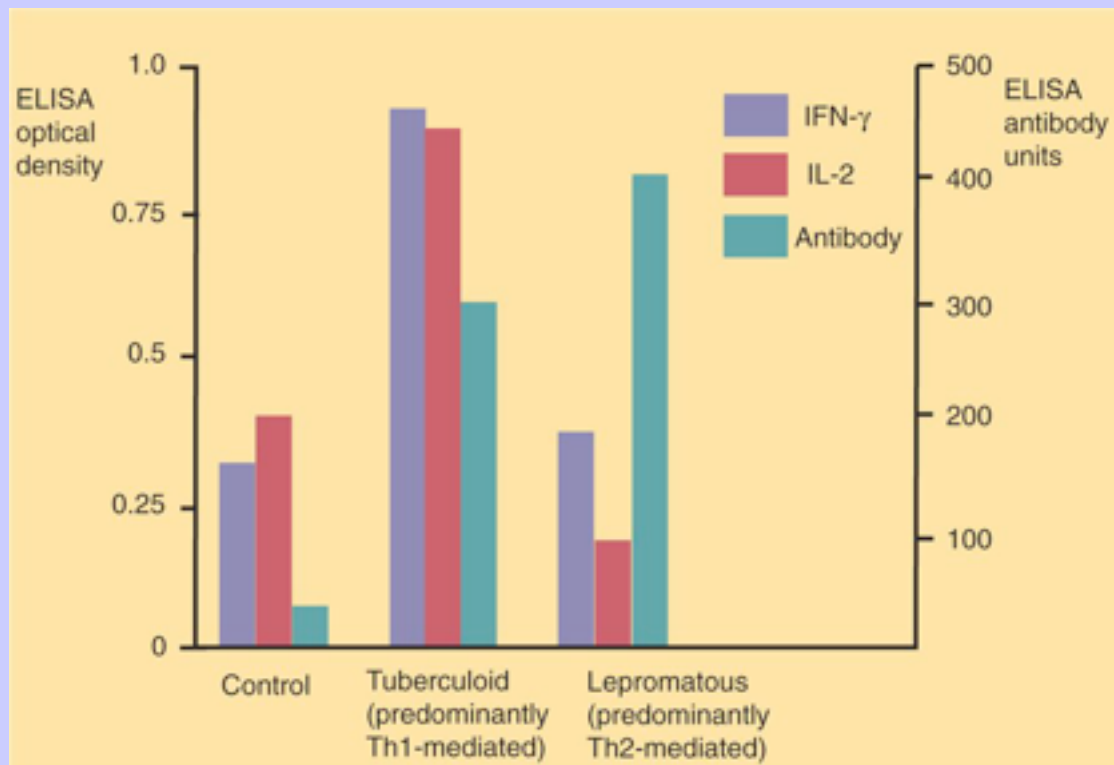


FIGURE 22-4 The differences in peripheral blood lymphocyte interleukin-2 (*IL-2*), interferon- γ (*IFN- γ*), and antibody production between sheep with the tuberculoid form and sheep with the lepromatous form of Johne's disease. Note that there is a marked tendency for the T cells from animals with the tuberculoid form of the disease to produce more Th1 cytokines than those with the lepromatous form. Despite this fact, animals with the latter form appear to produce more antibodies. (From data kindly provided by Dr. Chris Clarke and Mr. Charles Burrells.)



contain very few bacteria but large numbers of lymphocytes. Animals with the tuberculoid disease have T cells that produce more IL-2 and IFN- γ than sheep with the lepromatous form of the disease (Figure 22-4). In contrast, sheep with the lepromatous disease have slightly higher antibody levels. It is likely, therefore, that sheep with tuberculoid lesions mount an immune response in which Th1 cells predominate, whereas those with lepromatous disease employ Th2 cells.

IL-4 is the key cytokine that regulates the balance between Th1 and Th2 responses. For example, conventional IL-4 triggers Th2 responses while suppressing Th1 responses. It is apparent however, that animals can produce structural variants of IL-4. For example, in addition to normal IL-4, cattle produce two variants, called IL-4d2

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and IL-4d3, by alternative splicing of pre-mRNA. These variants may bind and block IL-4 receptors and so regulate its activity. As a result, these splice variants can influence the resistance of cattle to bovine tuberculosis and may account for the different responses to this infection. Animals showing significant resistance to tuberculosis produce high levels of IL-4d3 as compared to susceptible cattle. Like-wise transient increases in the ratio of IL-4d3 to IL-4 are observed following vaccination against bovine tuberculosis.

It must not be assumed from the above discussion that the specific Th subset involved in an immune response does not change once the response is established. Time-based studies have shown that immune responses to an organism can swing between Th1 and Th2 responses, perhaps several times, before a final response is established. This final response may well be a Th1 or Th2 response, or even some intermediate point in the Th1-Th2 spectrum. This variation appears to be a common feature of chronic infections such as tuberculosis.

22.3 EVASION OF THE IMMUNE RESPONSE

As pointed out previously, an invading microorganism becomes a pathogen because it can evade the immune defenses and survive within its host. On the other hand, bacteria, like all organisms, seek to avoid destruction. They have therefore evolved an incredibly diverse array of mechanisms by which they overcome host innate and acquired immune responses and evade elimination. These mechanisms are of two general types: they may avoid recognition by the immune system or, alternatively, they may seek to neutralize the host's immune effector mechanisms.

22.3.1 Prevention of Recognition

Campylobacter fetus ssp. venerealis, an organism that normally colonizes the genital tracts of cattle, prevents immune recognition by changing its surface coat. The destruction of most of these bacteria by a local immune response leaves some bacteria that possess new and different antigens. This residual population multiplies but is largely eliminated in turn by a second immune response, leaving organisms of a third antigenic type. This process of cyclical antigenic variation may be repeated for a long time, resulting in a persistent infection. *Anaplasma marginale*, a bacterium that lives within bovine red cells, also shows sequential antigenic variation. As a result, the number of *Anaplasma* in blood cycles at 6- to 8-week intervals. The number of bacteria gradually increases and then falls rapidly as a result of an antibody response. This is followed by development of a new antigenic variant that repeats the cycle. *A. marginale* is transmitted by ticks, so successful spread depends on maintenance of a high bacteremia.

TLR5 recognizes an evolutionary conserved site on bacterial flagellin. Some important motile bacteria such as *C. jejuni*, *H. pylori*, and *Bartonella bacilliformis* make an unusual form of flagellin that is not recognized by TLR5. These bacteria require motile flagellae in order to infect their mammalian hosts, so evasion of TLR5 is essential to their survival.

TLR9 recognizes unmethylated CpG dinucleotides found in bacterial DNA. But bacteria differ in the frequency of CpG dinucleotides in their DNA and, as a result, differ in their ability to activate TLR9. Bacteria that are potent stimulators of TLR9 signaling include *M. tuberculosis* and *Pseudomonas aeruginosa*. Bacteria that are weak TLR9 stimulators include *C. jejuni* and *Staphylococcus epidermidis*.

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22.3.2 Resistance to Effector Mechanisms

22.3.2.1 Evasion of Innate immunity

Many bacteria can block phagocytosis, Fc receptor function, cytotoxic T cell function, or complement activity. Many bacteria can survive within phagocytic cells. Some prevent recognition by phagocytic receptors. For example, *Staphylococcus aureus* inhibits its phagocytosis by expressing protein A on its surface. Protein A attaches to the Fc region of IgG molecules and so prevents antibodies from binding to Fc receptors on phagocytic cells or activating the classical complement pathway. *Taylorella equigenitalis* can bind equine IgG and IgM in a similar manner. *S. aureus* employs multiple mechanisms to prevent its destruction. For example, staphylokinase from *S. aureus* can bind and neutralize defensins. Another enzyme, aureolysin, destroys cathelicidins. *S. aureus* uses a cell wall peptidoglycan that is completely resistant to lysozyme, and all strains of *S. aureus* are catalase positive. Catalase can inactivate hydrogen peroxide and the free radicals produced during the respiratory burst. “Flesh-eating” *Streptococcus pyogenes* secretes a protease that destroys CXCL8 and so reduces the recruitment of neutrophils to its invasion sites.

Many bacteria can evade the complement system. Thus the M protein of streptococci can reduce opsonization by binding fibrinogen and so masking C3b binding sites. Streptococcal M protein can also bind factor H, thus inactivating bound C3b. *S. aureus* produces a protein that blocks C3 convertases and so inhibits complement-mediated opsonization. Other bacteria produce proteases that destroy complement components. *Salmonella* serotype *typhimurium* has a gene called Rck that confers resistance to complement-mediated lysis by preventing insertion of the MAC into the bacterial outer membrane.

Salmonellae possess cell-surface sensor molecules that serve as receptors for defensins. When they bind defensins they signal to the cell and alter the expression of genes that help combat the host's immune defenses. Thus the lipid A portion of lipopolysaccharide becomes modified by aminoarabinose and fatty acids in such a way that the negative charge and fluidity of the bacterial outer membrane are reduced. This results in decreased defensin binding and increased bacterial survival.

Bacteria such as enteropathogenic *E. coli*, *Yersinia pestis*, *M. tuberculosis*, and *P. aeruginosa* secrete molecules that depress neutrophil phagocytosis. In the case of *Pseudomonas*, for example, the bacterium uses a type III secretion system to inject toxins into the phagocytic cell. These toxins activate GTPases and disrupt intracellular signaling pathways. *Streptococcus canis* can inhibit chemotaxis and phagocytosis by producing toxins such as streptolysin O that lyse neutrophil cell membranes. Several Gram-negative bacteria of veterinary importance, such as *Mannheimia haemolytica* and *Fusobacterium necrophorum*, secrete leukotoxins. Leukotoxins kill leukocytes, especially granulocytes. They include several different molecules, but the most important are the RTX (“repeats in toxin”) proteins. For instance, *M. haemolytica* secretes an RTX toxin that kills ruminant neutrophils, alveolar macrophages, and lymphocytes. This leukotoxin binds to CD18 on leukocytes and, at low concentrations, induces their apoptosis via the intrinsic pathway. At high concentrations it produces transmembrane pores and necrosis. (This leukotoxin has been successfully incorporated in a vaccine against bovine respiratory disease.) *Moraxella bovis* also secretes a leukotoxin for bovine neutrophils. *Actinobacillus pleuropneumoniae* secretes a toxin that kills porcine macrophages. *Mycoplasma mycoides*, and possibly other mycoplasmas, can kill bovine T cells. The fungal toxin aflatoxin is also immunosuppressive, reducing the resistance of poultry to *Pasteurella multocida* and to *Salmonella* spp. Other bacteria trigger lymphocyte death by activating apoptotic pathways. These include *B. anthracis*, *Streptococcus* spp., *L. monocytogenes*, *S. aureus*, and *Yersinia* spp.

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Some bacteria can reduce the killing ability of phagocytes. For example, the carotenoid pigments responsible for the yellow color of *S. aureus* can quench singlet oxygen and permit the organism to survive the respiratory burst. *Salmonella* serotype *typhimurium* can prevent assembly of the NOX complex and downregulate host nitric oxide synthase 2 activity. *P. multocida* and *Histophilus somni* are also able to inhibit the respiratory burst.

22.3.2.2

Evasion of Acquired Immunity

Many pathogenic bacteria secrete proteases that can destroy immunoglobulins or cytokines. For example, proteases specific for IgA are produced by *Neisseria gonorrhoeae*, *Haemophilus influenzae*, and *Streptococcus pneumoniae*. These organisms can thus prevent opsonization and Fc receptor-mediated phagocytosis. *M. haemolytica* secretes a protease specific for bovine IgG1. *P. aeruginosa* secretes a protease that destroys IL-2. Monocytes obtained from cows subclinically infected with *Mycobacterium avium* ssp. *paratuberculosis* showed increased expression of IL-10 and suppressor of cytokine signaling-3 within two hours of exposure to these organisms. This response may reduce the ability of these monocytes to kill these microorganisms. T cells cultured in the presence of live *Salmonella* serotype *typhimurium* are prevented from proliferating and producing cytokines. These T cells fail to respond to *Salmonella*-infected dendritic cells. *B. abortus* produces a B cell mitogen (PrpA) that stimulates B cells to secrete IL-10. This causes transient immunosuppression and permits the bacterium to successfully establish a chronic infection. Finally, it must be pointed out that bacteria growing on a surface in a biofilm are much more resistant to opsonization and phagocytosis than single bacteria growing in suspension. The biofilm matrix appears to be able to inhibit opsonic killing.

22.4

ADVERSE CONSEQUENCES OF THE IMMUNE RESPONSES

Although immune responses are beneficial in that they eliminate invading bacteria, this is not always the case. The immune responses can influence the course of a bacterial disease without producing a cure and in some situations may increase its severity. The adverse consequences of the immune responses correspond in their mechanisms to the hypersensitivity types described in [Chapters 25](#) to [28](#). For example, a local type I hypersensitivity reaction is sometimes seen in sheep vaccinated against foot rot by means of *Dichelobacter nodosus* vaccine, but in this case it is believed that the hypersensitivity may assist in preventing reinfection.

Type II (cytotoxic) reactions may account for the anemia seen in animals with salmonellosis. In these infections, bacterial lipopolysaccharides from disrupted bacteria are adsorbed onto erythrocytes. The subsequent immune response against the bacterium and its products therefore results in red cell destruction. Although a similar anemia is observed in leptospirosis, its mechanism is unknown, since antibodies produced by infected animals may agglutinate normal red cells taken from the same animal before infection.

Type III (immune complex) reactions may contribute to the development of arthritis in *Erysipelothrix rhusiopathiae* infections in pigs or to the development of intestinal lesions in Johne's disease due to *M. avium* ssp. *paratuberculosis*. In the former case, bacterial antigen tends to localize in joints, where local immune complex formation then results in inflammation and arthritis. Passively administered antiserum may therefore exacerbate the arthritis in these infected animals. In Johne's disease, type I or type III reactions occurring in the intestinal mucosa may increase the outflow of fluid and diarrhea. It is probable, however, that the intestinal lesions in this disease are etiologically complex, since diarrhea can be transferred to normal calves by either plasma or leukocytes, and antihistamines may reduce the diarrhea. Type III hypersensitivity reactions are involved in purpura hemorrhagica of horses, in which immune complex lesions result from *Streptococcus equi* infection.

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Although cell-mediated immune responses are manifestly beneficial, they do contribute to the development of granulomatous lesions in some chronic infections. The development of large granulomas, although serving to wall off invading bacteria and so prevent their spread, may also involve uninfected tissues. If these granulomas invade essential structures such as airways in the lungs or large blood vessels, damage may be severe.

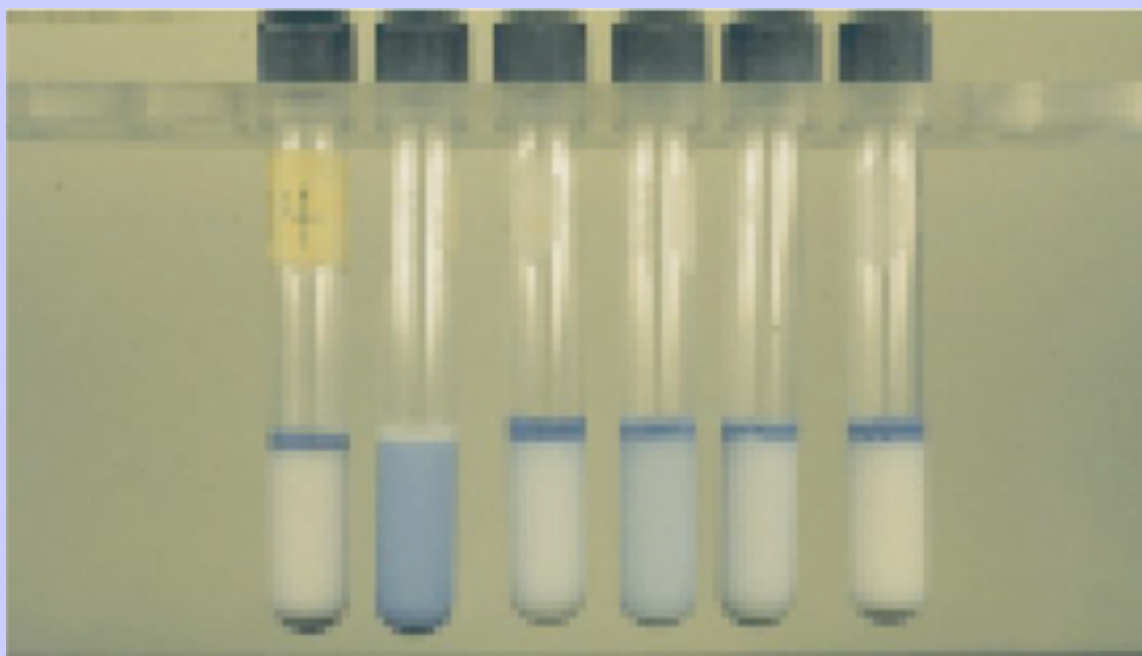
22.5 SEROLOGY OF BACTERIAL INFECTIONS

Bacterial infections are often diagnosed by detecting the presence of specific antibodies in serum. Thus the agglutination test is widely employed in the diagnosis of bacterial infections, particularly those involving Gram-negative bacteria such as *Brucella* spp. and *Salmonella* spp. The usual procedure in bacterial agglutination tests is to titrate serum (antibody) against a standard suspension of antigen. Bacteria are not, of course, antigenically homogeneous but are covered by a mosaic of many different antigens. Thus motile bacteria will have flagellar (H) antigens, and agglutination by anti-flagellar antibodies

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FIGURE 22-5 The milk ring test. Stained *Brucella* remains suspended in the milk in a negative test but rises with the cream in a positive reaction (second tube). (Courtesy Dr. John Huff.)



will produce fluffy cotton-like floccules as the flagella stick together, leaving the bacterial bodies only loosely agglutinated. Agglutination of the somatic (O) antigens results in tight clumping of the bacterial bodies so that the agglutination is finely granular. Many bacteria possess several O and H antigens, as well as capsular (K) and pilus (F) antigens. By using a set of specific antisera, it is possible to characterize the antigenic structure of an organism and consequently to classify it. It is on this basis, for instance, that the 2400 or so different serovars of *S. enterica* are classified.

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Flagella (H) antigens are destroyed by heating, whereas O antigens are heat-resistant and therefore remain intact on heat-killed bacteria. K antigens vary in their heat stability: The L antigen of *E. coli*, which is a capsular antigen, is heat labile, whereas another K antigen, antigen A, is heat stable. *Salmonella* serotype *typhi* possesses an antigen called Vi that, although heat stable, is removed from the bacterial cells by heating. The presence of K or Vi antigens on an organism may render them O-inagglutinable and thus complicate agglutination tests. It should also be pointed out that rough forms of bacteria do not form stable suspensions and therefore cannot be typed by means of agglutination tests.

Bacterial agglutination tests may be performed by mixing drops of reagents on glass slides or by titrating the reagents in tubes or wells in plastic plates. Tube agglutination tests are commonly used for such diseases as salmonellosis, brucellosis, tularemia, and campylobacteriosis. Slide agglutination tests are commonly used as screening tests. These include the *Brucella*-buffered antigen tests, in which killed, stained *Brucella* organisms are suspended in an acid buffer (pH 3.6). The dye used, either the red dye rose-bengal or a mixture of crystal violet and brilliant green, enables the test to be easily read. At this low pH, nonspecific agglutination by IgM antibodies is eliminated. The *Brucella*-buffered plate agglutination test has a specificity of as high as 99% and a sensitivity of 95%. The efficient and widespread use of these tests has eliminated bovine brucellosis from many countries.

Salmonella serotype *pullorum* infection in poultry can be diagnosed by a slide agglutination test, in which killed bacteria stained with gentian violet are mixed with whole chicken blood. Agglutination is readily seen if antibodies are present. Leptospirosis is diagnosed by a microscopic agglutination test, in which mixtures of living organisms and test serum are examined under the microscope for agglutination. This technique preferentially detects IgM antibodies and is thus an excellent test for detecting recent outbreaks, as well as for distinguishing between infected and vaccinated animals.

It is not mandatory that serum be used as the source of antibody for diagnostic tests. The presence of antibodies in body fluids other than serum, such as milk whey, vaginal mucus, or nasal washings, may be of more significance, especially if the infection is of a local or superficial nature. One such example is the milk ring test used to detect the presence of antibodies to *B. abortus* in milk (Figure 22-5). Fresh milk is shaken with bacteria stained with hematoxylin or triphenyl tetrazolium and is allowed to stand. If antibodies, especially those of the IgM or IgA classes, are present, the bacteria will clump and adhere to the fat globules of the milk and rise to the surface with the cream. If antibodies are absent, the stained bacteria will remain dispersed in the milk, and the cream, on rising, will remain white.

22.6 IMMUNITY TO FUNGAL INFECTIONS

Fungal infections fall into three patterns: (1) primary infections by fungi that affect the skin or other surfaces such as *Microsporum* spp. or *Candida* spp.; (2) primary infections by dimorphic fungi that largely cause respiratory infections, for example, *Histoplasma capsulatum*, *Blastomyces dermatitidis*, and *Coccidioides immitis*; and (3) secondary infections by opportunistic fungi in immunodeficient animals, for example the Mucorales (*Rhizopus* spp., *Mucor* spp., or *Absidia* spp.). Primary infections are combated by both innate and acquired immune mechanisms. Thus innate immune mechanisms against invasive fungi such as *Candida* spp. or *Aspergillus* spp. include activation of the alternate pathway of the complement system resulting in attraction of neutrophils and attempts by these neutrophils to ingest the invading hyphae or pseudohyphae. Neutrophils are also activated by the IL-23/IL-17 axis during fungal infections. Thus fungal pathogen-associated molecular patterns acting either through TLR2 or through a cell-surface lectin called dectin-1 turn on IL-23 synthesis. IL-23 activates Th17 cells.

The IL-17 produced by these Th17 cells then activates both neutrophils and endothelial cells and promotes acute inflammation. It is of interest to note that culturing T cells and monocytes in the presence of *Candida* hyphae

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promotes the generation of Th17 cells. In contrast, culture in the presence of the *Candida* yeast form promotes production of IL-12 and a Th1 response. Because of their size, neutrophils cannot totally ingest the invading fungi. Nevertheless, by releasing their enzymes into the tissue fluid, neutrophils may severely damage fungal hyphae. Very small fungal fragments or spores may be ingested and destroyed by macrophages or by NK cells.

Once established, fungal infections can be destroyed only by Th1-mediated mechanisms. Thus some species of *Aspergillus* are facultative intracellular parasites, and chronic or progressive fungal diseases are commonly associated with defects in the T cell system. Th1 functions primarily in fungal infections by activating macrophages and by promoting epidermal growth and keratinization. Some T and NK cells can exert a direct cytotoxic effect on yeasts such as *Cryptococcus neoformans* and *Candida albicans*. It is not uncommon for recovered animals to develop a type IV hypersensitivity to fungal antigens. The critical importance of acquired immunity to fungi is seen in the way that fungal infections, such as those caused by *Pneumocystis carinii*, develop in immunosuppressed individuals such as AIDS patients.

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23 CHAPTER 23 Acquired Immunity to Viruses

23.1 KEY POINTS

- When sentinel cells detect foreign nucleic acids through their toll-like receptors and other receptors, they are triggered to secrete the antiviral type I interferons.
- Antibodies are effective against extracellular viruses and can prevent them from binding to cells and thus neutralize them.
- Cell-mediated responses are primarily responsible for antiviral immunity. The major mechanism involved is the killing of virus-infected cells by cytotoxic T cells.
- Because viruses are obligate intracellular parasites, they employ a wide variety of methods of evading the immune response.
- Some viruses may cause minimal disease themselves, but significant damage and disease may be caused by the immune response to these viruses.

Since viruses are obligate intracellular organisms, their very existence is threatened if they are destroyed by the immune system or by the death of their host. Because of these opposing factors, both viruses and their hosts have been subjected to rigorous selection and adaptation. Viruses are selected for their ability to evade the host's immune responses, while at the same time animals are selected for resistance to virus-induced disease. Viruses that are eliminated before they replicate cannot spread. Hosts eliminated by viruses can no longer serve as hosts. An “over-successful” virus will reduce the availability of susceptible hosts, while a very successful host will be the largest target for the next generation of viruses. As a result, there can never be a “solution” to the problem of viruses. Virus diseases therefore tend to be lethal when the virus first encounters its host species or infects the wrong species. However, once viruses and their hosts have interacted for a long period, any resulting disease tends to become increasingly mild.

For example, in infections in which virus-host adaptation is poor, diseases tend to be acute and severe. Rabies is an excellent example of this. The virus is inevitably lethal in dogs, cats, horses, and cattle because they are unnatural hosts. On the other hand, in its natural hosts, especially bats and skunks, rabies virus persists and can be shed in saliva for a long period without causing disease. From the virus's “point of view,” infection of dogs, cattle, or horses is unprofitable since those animals almost never transmit rabies to skunks. Other diseases of this type include feline panleukopenia, canine parvovirus-2, and the virulent forms of Newcastle disease. Vaccination is relatively successful in this type of infection since the virus has not adapted to the host's defenses.

When the virus and its host are more adapted, although disease may be severe, mortality may not be high and the virus may be persistent. In this type of disease, further attacks may occur as a result of infection by antigenic variants of the same virus. Examples of this type of virus infection include foot-and-mouth disease and influenza. Vaccination against diseases of this type is complicated by the antigenic diversity among viruses circulating in the population.

Even more-adapted viruses can result in persistent infection, where the immune system is unable to eliminate the virus. Diseases of this type include the lentivirus infections, equine infectious anemia (EIA), maedi-visna of sheep, and AIDS in humans. The virus may even change during these infections and thus constantly evade the immune system. Vaccination against these diseases is essentially unsuccessful. As their adaptation increases, viruses may cause latent infections and relatively mild, nonlethal disease. Some herpesvirus infections fall into this category. The

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most extreme examples of virus adaptation are those in which the viral genome becomes stably integrated into the host genome. These endogenous viruses are well recognized in domestic mammals such as the cat and pig.

In studying the nature of the host responses to viruses, it is well to recognize that this continuing selective pressure on both host and virus exists and profoundly influences the outcome of all viral infections.

23.2 VIRUS STRUCTURE AND ANTIGENS

Virus particles, called virions, consist of a nucleic acid core surrounded by a layer of protein or lipoproteins (see [Chapter 7, Figure 7-2](#); and [Chapter 35, Figure 35-1](#)). This protein layer is called the capsid; its subcomponents are called capsomeres. An envelope containing lipoprotein derived in part from the host cell may also surround virions. The complexity of virions varies. Some, such as poxviruses, are complex, whereas others, such as foot-and-mouth disease virus (FMDV), are relatively simple. Antibodies can be produced against epitopes on all the proteins situated inside and on the surface of the virion. Antibodies against the nucleoprotein components are not usually significant from a protective point of view, but they may be useful in diagnosis.

23.3 PATHOGENESIS OF VIRUS INFECTIONS

Adsorption, the first step in the invasion of a cell by a virus, occurs when a virion binds to receptors on the cell surface. These receptors are not designed for the convenience of viruses but have some other physiological function in normal cells. Thus the rabies virus binds to the receptor for acetylcholine, a neurotransmitter. The Epstein-Barr virus (the cause of infectious mononucleosis) binds to a receptor for C3. Rhinoviruses that cause the common cold bind to cell-surface integrins. The chemokine receptor CCR5 has been identified as the receptor used by West Nile virus. The nature, number, and distribution of host cell receptors determine the host range and tissue tropism of a virus. The bound virion is taken into the cell through endocytosis or by fusion with the plasma membrane. Once inside a cell, the virus capsid is dismantled so that its nucleic acid is released into the cell cytoplasm—a process called uncoating. Once the virus genome is uncoated, it begins the process of replication ([Figure 23-1](#)). The host cell DNA, RNA, and protein synthesis are usually inhibited so that only the viral genetic information is processed. The site within the cell where this happens differs among viruses and depends on their nucleic acid. If the virus—for example, a herpesvirus—contains DNA, this viral DNA is replicated. The new viral DNA is then transcribed into viral messenger RNA, and this RNA is translated into new capsid proteins. The new capsid proteins are assembled into new virions. The host cell also replicates the viral nucleic acid so that large quantities of viral DNA are produced. This viral DNA is packaged inside the new capsid so that complete virions are formed. If the virus is unenveloped, the infected cells disintegrate and the virions are released into the environment. If the virions are enveloped, they leave the cell by budding through the cell surface. The cell membrane that encloses them serves as the new envelope. The released virions may then spread to nearby cells and invade them.

If a virus contains RNA rather than DNA, its replication takes a slightly different course. For most RNA viruses, such as Newcastle disease or FMDV, viral DNA is not used. Thus in FMDV infection, the viral single-stranded RNA (the “plus strand”) is used as a template to synthesize a complementary “minus strand” of RNA. These minus strands are then used to generate new plus strands that can be translated into viral proteins. Some viruses contain double-stranded RNA and use only one of the strands generated during replication. In other RNA viruses, the infecting virus RNA may be complementary to the newly synthesized viral RNA that will translate into viral proteins.

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FIGURE 23-1 The mechanism of replication of DNA and RNA viruses.

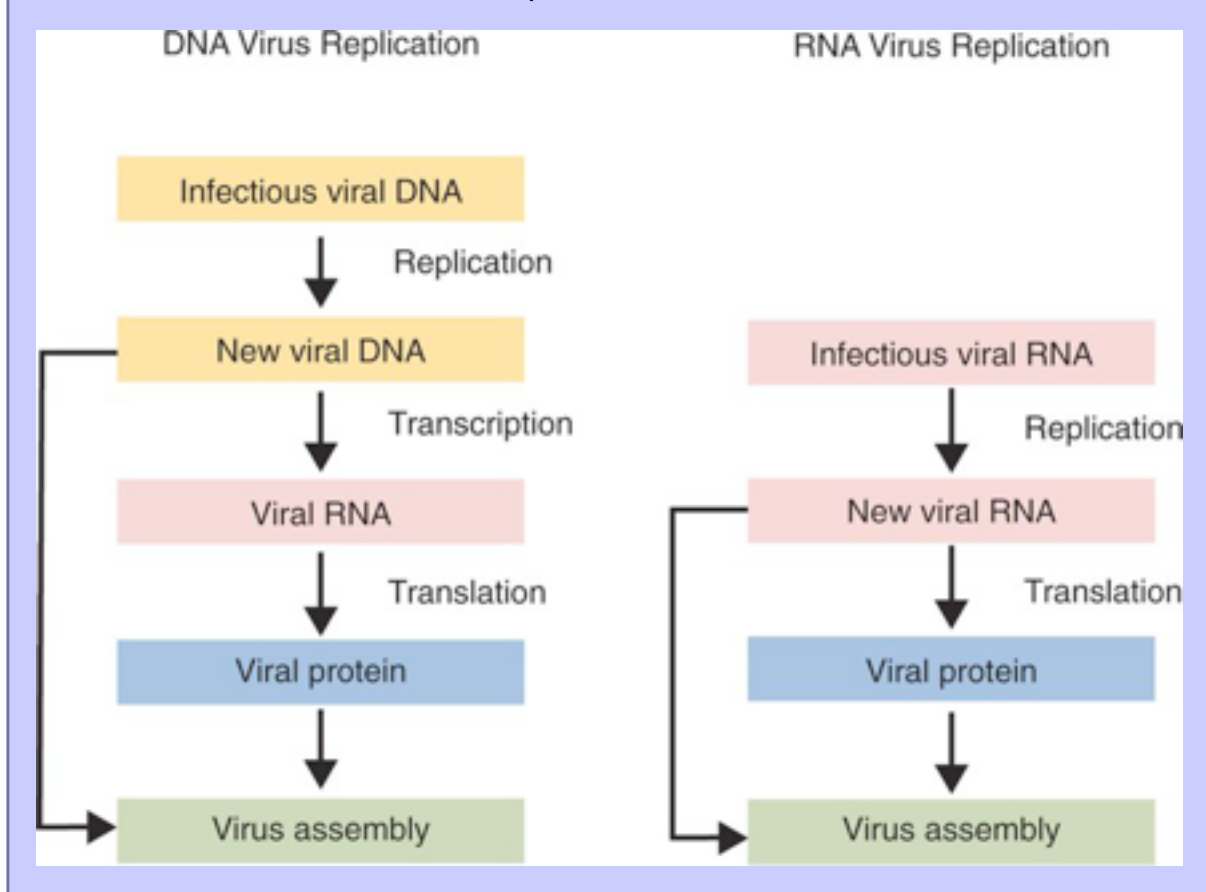
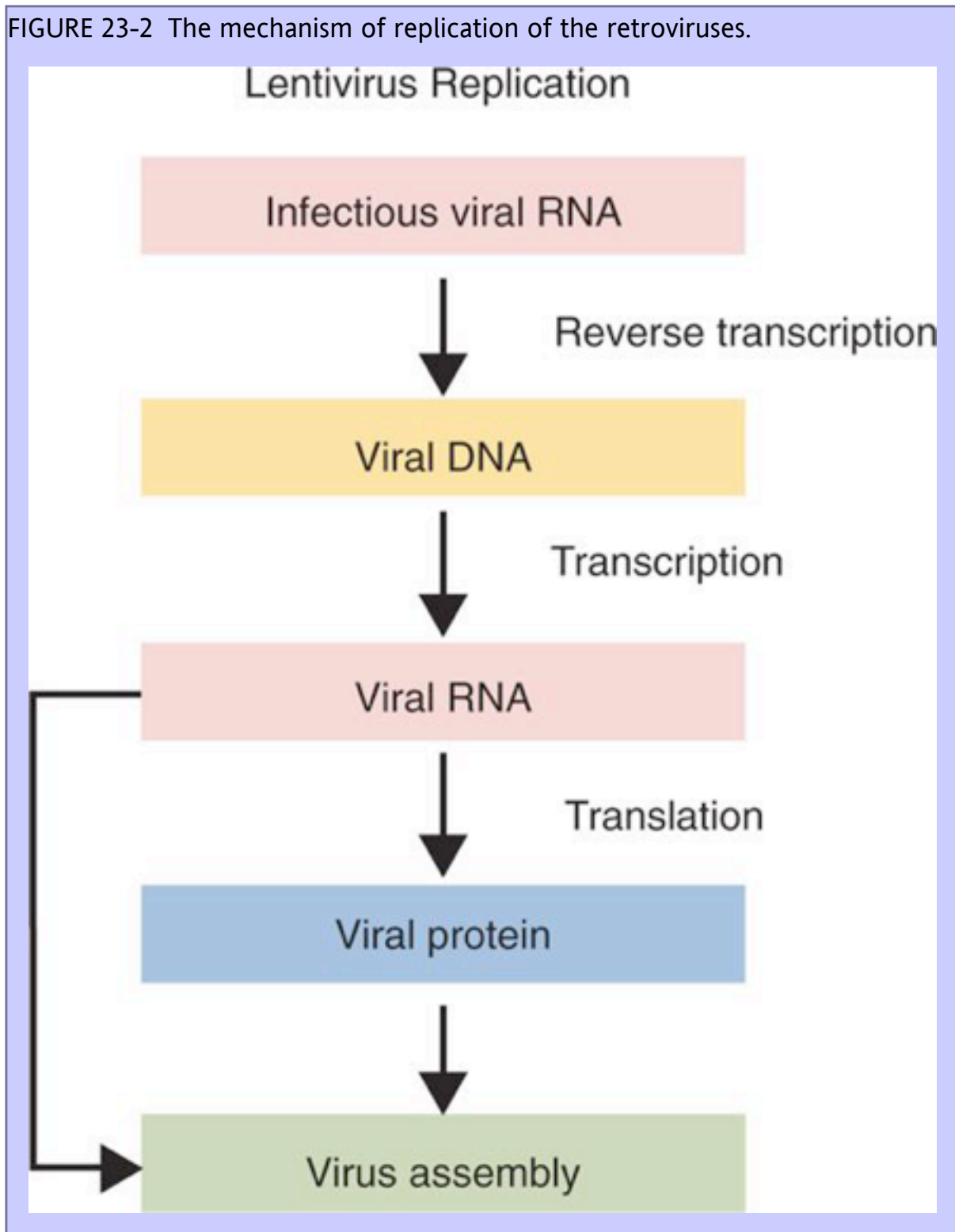


FIGURE 23-2 The mechanism of replication of the retroviruses.



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A different replication mechanism is employed in the case of some RNA tumor viruses and immunodeficiency viruses ([Figure 23-2](#)). These are called retroviruses since their RNA is first reversely transcribed into DNA. To do this, they use an enzyme called a reverse transcriptase. The new viral DNA moves into the cell's nucleus and is integrated into the host cell genome as a provirus. This proviral DNA can then be transcribed into RNA, as well as being able to copy itself. The proteins and RNA can then be packaged into complete new virion.

Changes in virus-infected cells may be minimal, perhaps only detectable by the development of new proteins on the cell surface. Sometimes, however, the changes may be extensive and result in either cell lysis or malignant transformation and the development of tumors.

23.4 INNATE IMMUNITY

Rapid, powerful innate immune responses limit many viral infections by inhibiting viral replication. Interferons are especially important to viral resistance. Lysozyme can destroy several viruses, as can many intestinal enzymes and bile. Collectins bind to viral glycoproteins and block virus interaction with host cells. For example, conglutinin, mannose-binding lectin, surfactant protein-A (SP-A), and SP-D have all been shown to inactivate influenza viruses. Defensins from leukocytes and mucosal epithelial cells play a dual role in antiviral defenses since they can act both on the virus and on the host cell. Thus defensins can inactivate enveloped virions by disrupting their envelopes or by interacting with their glycoproteins. Some defensins can act on virus-infected cells by blocking intracellular signaling pathways and interfering with transcription of viral RNA. Finally, cells invaded by viruses may undergo premature apoptosis, thus preventing successful viral invasion and replication.

23.4.1 Antiviral Cytokines

The interferons are cytokines secreted by virus-infected cells that protect other cells against viral, bacterial, and protozoan invasion. They are glycoproteins with molecular weights of 20 to 34 kDa. They fall into two major types, type I and type II interferons. The type I interferons include interferon- α (IFN- α). IFN- α is produced in large quantities by plasmacytic dendritic cells and in much smaller amounts by lymphocytes, monocytes, and macrophages. Most mammals produce multiple isoforms of IFN- α . (There are 18 different isoforms in humans, 12 in pigs and cattle, 4 in horses, and 2 in dogs.) IFN- β is derived from virus-infected fibroblasts. (There are five isoforms in cattle and pigs, and one in dogs and humans.) IFN- ω is produced by lymphocytes, monocytes, and human, horse, pig, rabbit, and dog trophoblast cells (six to seven in pigs, five in humans, two in horses, and zero in dogs). (IFN- ω is also called IFN- α II.) A distinct form of type I interferon, IFN- τ , has been isolated from the ruminant trophoblast, and IFN- δ has been isolated from the pig trophoblast. IFN- δ is only distantly related to the other type I interferons. There are two forms of IFN- κ (interleukin-28 [IL-28] and IL-29). There is only one type II interferon, IFN- γ , a cytokine derived primarily from antigen-stimulated T cells. It is also produced in pig trophoblast cells. The type I interferons are stable at pH 2, whereas IFN- γ is not ([Box 23-1](#)).

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In most cases these molecules probably act on virus-infected cells to inhibit viral growth. The trophoblast interferons also regulate a mother's immune responses to her fetus. The receptor complex for both IFN- α and IFN- β signals through JAK/STAT proteins to activate the genes coding for multiple antiviral proteins (see [Chapter 6](#), [Figure 6-8](#)).

The two type I interferons α/β are produced by virus-infected cells within a few hours after viral invasion, and high concentrations of interferons may be achieved in vivo within a few days, long before acquired immunity develops. For example, in cattle infected intravenously with bovine herpesvirus-1, peak interferon levels in

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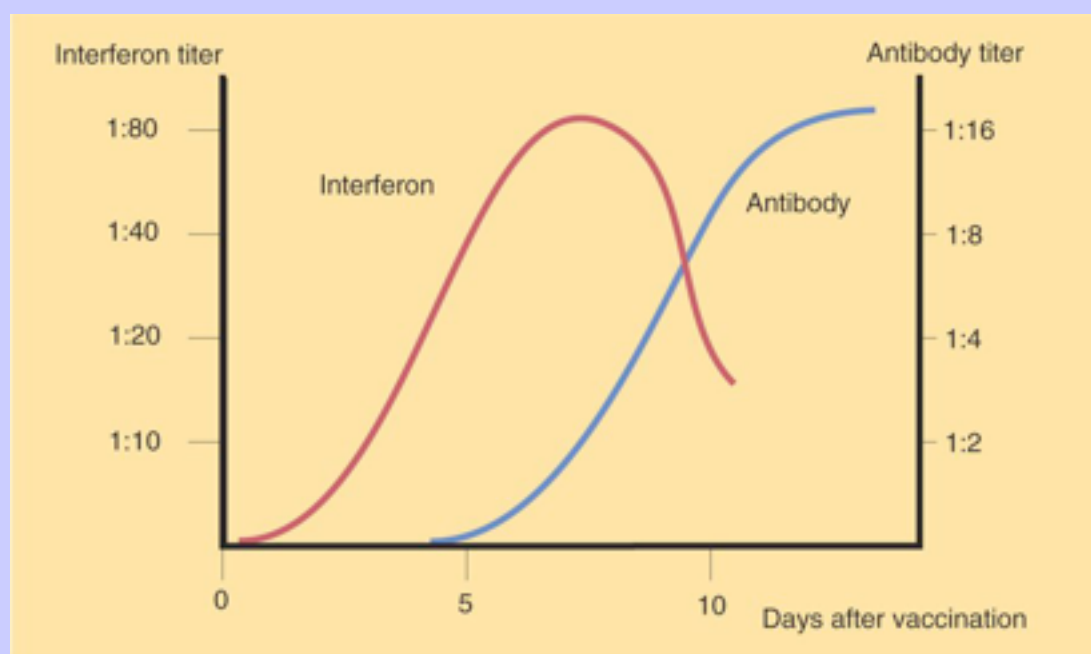
serum are reached 2 days later and then decline, but they are still detectable by 7 days ([Figure 23-3](#)). In contrast, antibodies are not usually detectable in serum until 5 to 6 days after the onset of a virus infection.

23.4.1.1

Interferon Induction

Viruses, unlike bacteria and fungi, do not contain easily recognizable microbe-specific structures since they are constructed from host-derived components. For this reason, animal cells have evolved the ability to recognize their only virus-specific components, their nucleic acids. Two complementary receptor systems recognize viral nucleic acids. One system consists of nucleic acid sensor proteins found within the cytoplasm of all nucleated cells. These sensor proteins are called RIG-1 and MDA5. These molecules

FIGURE 23-3 The sequential production of interferon and antibody following intranasal vaccination of calves with infectious bovine rhinotracheitis vaccine. (From data kindly provided by Dr. M. Savan.)



detect double-stranded viral RNA produced by viral infection and then signal through several adaptor proteins to activate the IFN- β gene. There is also evidence for the existence of a cytoplasmic system to detect viral DNA. The second system is mediated by toll-like receptors 3 (TLR3), 7, 8, and 9. For example, TLR3 recognizes double-stranded RNA. TLR7 and TLR8 recognize single-stranded RNA viruses such as vesicular stomatitis virus and influenza virus. TLR9 detects unmethylated CpG motifs in DNA. These motifs are common in both DNA viruses and bacteria. Mice deficient in either TLR7 or TLR9 or their adaptor protein MyD88 show reduced ability to defend themselves against viruses. Plasmacytoid dendritic cells have a specialized signaling pathway that links TLR7 and TLR9 to the production of very large amounts of type I interferons.

23.4.1.1.1

Box 23-1 Measuring Interferons

Interferons may be assayed by measuring their antiviral effects. For example, serum samples to be tested for interferon activity are added to fibroblast cultures at various dilutions and incubated for 18 to 24 hours. The fibroblast monolayers are then washed, and a standard quantity of bovine vesicular stomatitis virus is added to each culture. After 48 hours of incubation, the monolayers are stained and virus plaques are seen as cleared areas in the monolayer. The presence of interferons in the test serum will reduce the number of plaques formed. This is called a plaque reduction assay.

23.4.2

Type I Interferons

23.4.2.1

Antiviral Activities

The type I interferons are secreted by virus-infected cells and bind to CDw118 on infected and nearby cells in an autocrine and paracrine manner. Receptor binding results in the development of “an antiviral state” within a few minutes that peaks 5 to 8 hours later. The interferons signal to the cell and stimulate the activation of hundreds of genes ([Figure 23-4](#)). Some of these new proteins have antiviral activity. For example, type I interferons upregulate transcription of 2'5'-oligoadenylate synthetase (2'5'OAS) genes. Expressed OAS enzymes are then converted from an inactive to an active form by exposure to dsRNA. The activated enzymes act on adenosine triphosphate (ATP) to form 2'5' adenylyate oligomers. These oligomers in turn activate a latent ribonuclease called RNAase L ([Figure 23-5](#)). RNAase L degrades viral RNA and so inhibits viral growth. Another antiviral protein induced by IFN- γ , especially in activated macrophages, is nitric oxide synthase. The nitric oxide generated by this enzyme prevents virus growth in interferon-activated macrophages. (It also increases resistance to some bacteria.) Another antiviral factor induced by interferons is a protein kinase called PK-P1. PK-P1 phosphorylates an initiation factor called eIF2a, which then prevents translation initiation of viral mRNA.

While many of the molecules induced by type I interferons have very nonspecific antiviral activity, others are specific for distinct viral classes. For example, Mx proteins are GTPases that inhibit the translation of influenza virus mRNA by suppressing G-protein activity (see [Chapter 6](#)).

The ability of cells to produce interferon varies. Virus-infected leukocytes, especially plasmacytic dendritic cells, produce large amounts of IFN- α ; virus-infected fibroblasts produce IFN- β ; and antigen-stimulated T cells are the major source of IFN- γ (see [Chapter 12](#)). Kidney cells are poor interferon producers, and neutrophils produce no interferon. Although live or inactivated viruses are the most important stimulators of interferon production, interferons may

FIGURE 23-4 The receptor for the type I interferons. Ligand binding triggers the Janus kinase (JAK)–signal transducer and activator of transcription (STAT) transduction pathway and eventually activates both antiviral and immunoregulatory pathways.

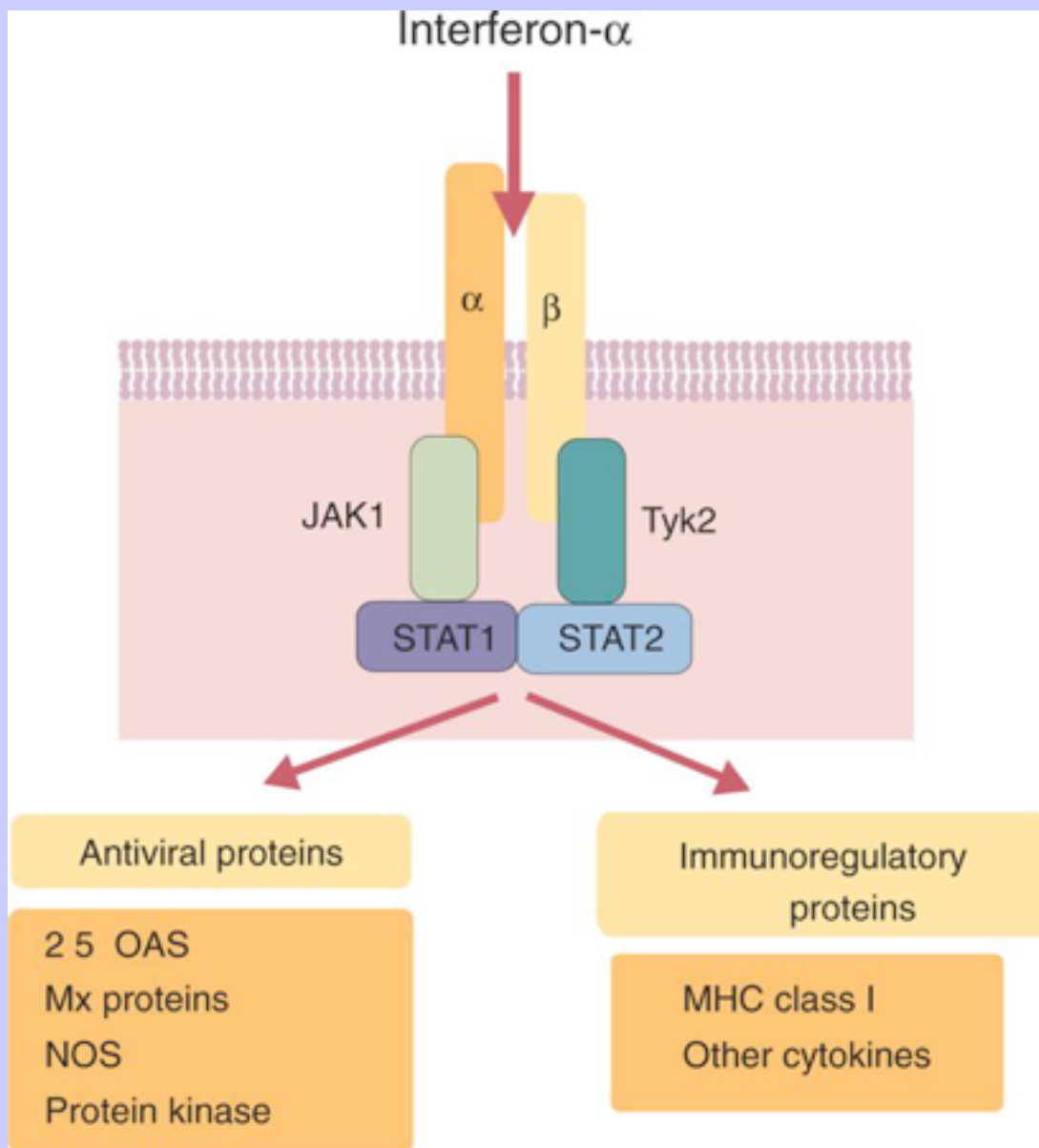
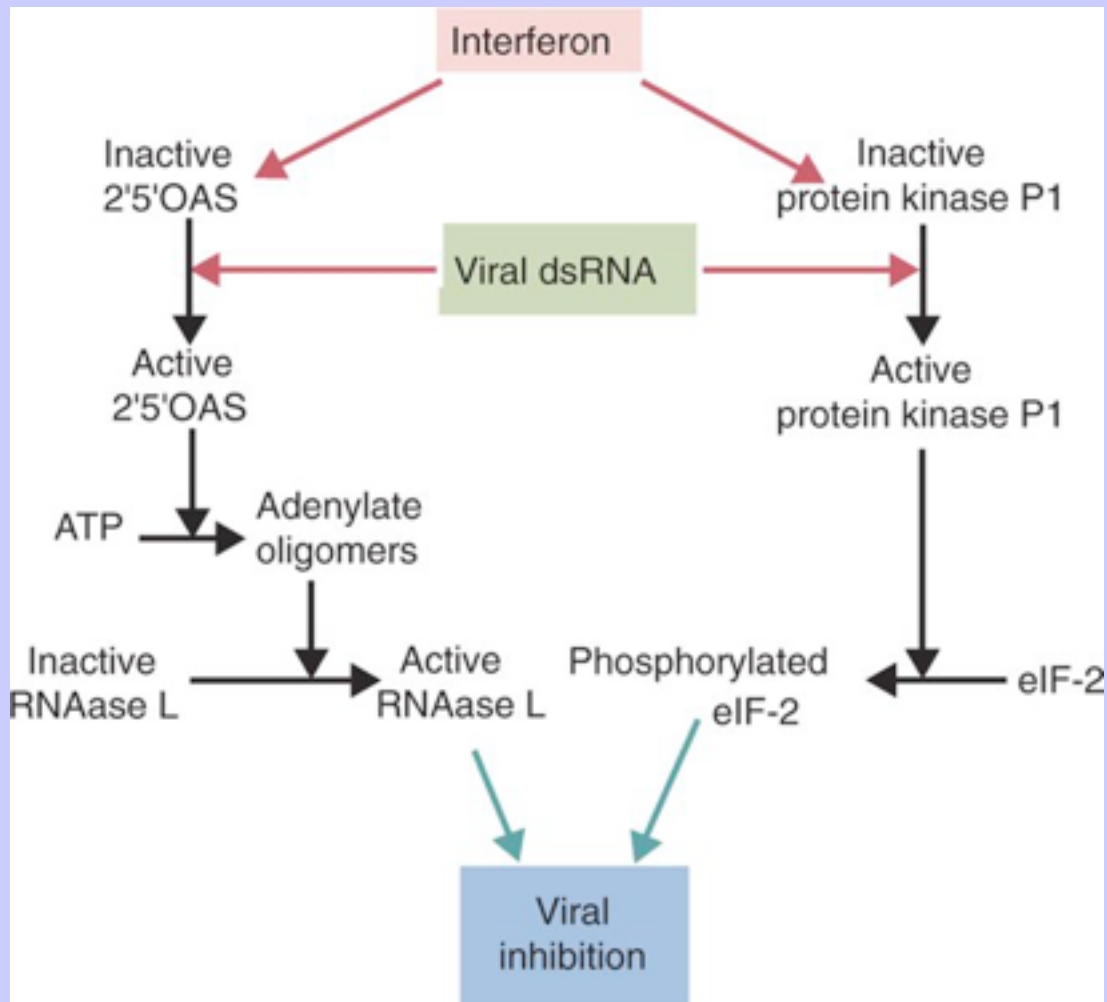


FIGURE 23-5 Some of the mechanisms by which the interferons can exert their antiviral activities.



also be induced by lipopolysaccharides by binding to TLR4.

Lymphocytes from normal, unsensitized donors can kill virus-infected cells. This innate cytotoxicity is due to natural killer (NK) cells (see [Chapter 30](#)). NK cell cytotoxicity is stimulated by type I interferons and, as a result, is enhanced early in a virus infection. NK cells also produce IFN- γ , and this too has a direct antiviral effect. NK cells may therefore reduce the severity of viral infections long before the development of acquired immunity and the appearance of specific cytotoxic T cells.

IFN- α activates NK cell-mediated cytotoxic activity, and it stimulates the differentiation of monocytes into dendritic cells, as well as the maturation and activity of dendritic cells. IFN- α also participates in the transition from innate to acquired immunity and drives certain γ/δ T cell responses. It stimulates memory T cell proliferation, it activates naïve T cells, and it enhances antigen-specific T cell priming.

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23.5 ACQUIRED IMMUNITY

23.5.1 Antibody-Mediated Immunity

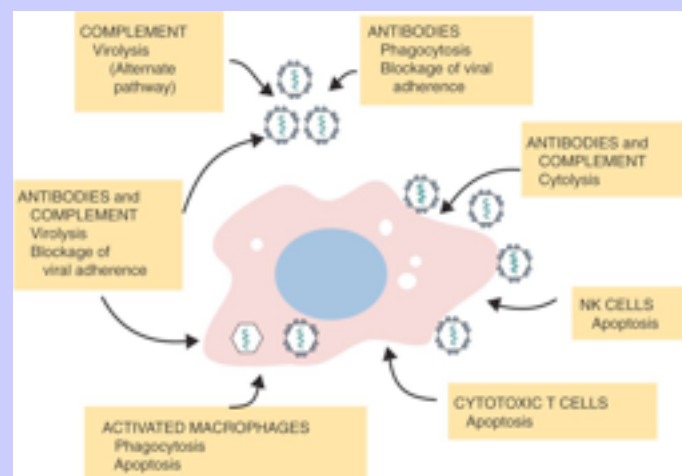
Virus capsids are antigenic, and it is against these and the envelope proteins that antiviral immune responses are largely mounted ([Figure 23-6](#)). Antibodies can prevent cell invasion by blocking the adsorption of virions to target cells, by stimulating phagocytosis of viruses, by triggering complement-mediated virolysis, or by causing viral clumping and so reducing the number of infectious units available for cell invasion. The combination of antibody and virus does not destroy a virus, since the splitting of virus-antibody complexes may release infectious virions.

Antibodies are not only directed against proteins on free virions, but they are also directed against viral antigens expressed by infected cells. As a result, these infected cells may also be destroyed. Virus infections in which antibody-mediated destruction of infected cells occurs include Newcastle disease, rabies, bovine viral diarrhea, infectious bronchitis of birds, and feline leukemia. Antibodies may kill infected cells by complement-mediated cytotoxicity or by antibody-dependent cell-mediated cytotoxicity (ADCC). These cytotoxic cells include lymphocytes, macrophages, and neutrophils with Fc receptors through which they can bind to antibody-coated target cells.

The immunoglobulins that neutralize viruses include immunoglobulin G (IgG) and IgM in serum and IgA in secretions. IgE may also play a protective role, since humans with an IgE deficiency suffer from severe respiratory infections. As in antibacterial immunity, IgG is quantitatively the most significant immunoglobulin, whereas IgM is qualitatively superior.

Although most viruses infect cells by binding directly to receptors on target cells, some use an intermediate molecule. For instance, some antibody-coated viruses bind to cells through Fc receptors. This, of course, facilitates endocytosis of the virus and may thus enhance virus infection. Complement may enhance some virus infections in a similar fashion.

FIGURE 23-6 The ways in which the immune system can protect the body against viruses.



Examples of viruses whose infections are enhanced by antibodies include feline infectious peritonitis (FIP), Aleutian disease of mink, African swine fever, and human immunodeficiency virus.

23.5.2

Cell-Mediated Immunity

Although antibodies and complement can neutralize free virions and destroy virus-infected cells, cell-mediated immune responses are much more important in controlling virus diseases. This is readily seen in immunodeficient humans (see [Chapter 34](#)). Those who cannot mount an antibody-mediated response suffer from overwhelming bacterial infections but tend to recover from the common viral diseases. In contrast, humans with a T cell deficiency are commonly resistant to bacterial infection but highly susceptible to virus diseases.

Viral antigens may be expressed on the surface of infected cells long before progeny viruses are produced. When this endogenous antigen is presented by major histocompatibility complex (MHC) class I molecules, virus-infected cells are recognized as foreign and killed. Viruses require host cells in which to replicate. Elimination of infected cells prevents viral spread. Although antibody and complement or ADCC can play a role in this process, T cell-mediated cytotoxicity is the major destructive mechanism. Cytotoxic T cells recognize the peptide-MHC complexes and kill them. Type I interferons can sensitize virus-infected cells to this cytotoxic effect. Under some circumstances, cytotoxic T cells may kill intracellular viruses without killing the infected cells. This antiviral effect is mediated by T cell-derived IFN- γ and tumor necrosis factor- α (TNF- α). These cytokines activate two virucidal pathways. One pathway eliminates viral nucleocapsid particles, including their contained genomes. The second pathway destabilizes viral RNA.

Some viral antigens may function as superantigens by binding directly to T cell antigen receptor V_{β} chains. For example, rabies virus nucleocapsid binds to mouse $V_{\beta}8$ T cells. By stimulating helper T cell activity, rabies viruses can switch on Th2 cells. This in turn can result in an enhanced immune response to rabies viruses, as well as a polyclonal B cell response sometimes seen in this disease.

Macrophages also develop antiviral activity following activation. Viruses are readily endocytosed by macrophages and are usually destroyed. If the viruses are noncytopathic but can, however, grow inside macrophages, a persistent infection may result. Under these circumstances, the macrophages must be activated in order to eliminate the virus. Thus immunity mediated by IFN- γ is a feature of some virus diseases (see [Chapter 16](#)). For example, macrophages from birds immunized against fowlpox show an enhanced antiviral effect against Newcastle disease virus and will prevent the intracellular growth of *Salmonella enterica gallinarum*, a feature that is not a property of normal macrophages.

The duration of immunological memory to viruses is highly variable. Thus, antibodies against viruses may persist for many years in the absence of the virus. On the other hand, because of the hazards of persistent cytotoxicity, cytotoxic T cells die soon after virus elimination. Memory T cells can, however, persist for many years.

23.6

EVASION OF THE IMMUNE RESPONSE BY VIRUSES

As discussed at the beginning of this chapter, during the millions of years they have coexisted with vertebrates, viruses have evolved to manipulate host immune responses. As a result, the relationship between host and virus must be established on the basis of mutual accommodation so that the long-term survival of both is ensured. Failure to reach this accommodation will result in the elimination of either host or virus, and death of the host automatically eliminates the virus.

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Viruses in different families use different survival strategies. Thus RNA viruses have a very small genome with little room to spare for genes dedicated to suppressing immunity. The proteins of RNA viruses therefore tend to be multifunctional. On the other hand, DNA viruses have a larger genome and can afford to devote many different genes to immune evasion. In DNA viruses such as the poxviruses and herpes-viruses, as much as 50% of the total genome may be devoted to immunoregulatory genes.

23.6.1 Inhibition of Humoral Immunity

One of the simplest mechanisms of evading destruction involves antigenic variation. The most significant examples of this occur among the influenza A viruses and the lentiviruses.

Influenza A viruses possess envelope proteins called hemagglutinins and neuraminidases. There are 13 different hemagglutinins and 9 neuraminidases found among the type A influenza viruses; they are identified according to a standard nomenclature system. Thus the hemagglutinin of the swine influenza virus is called H1, and its neuraminidase is called N1. The two subtypes of the equine influenza viruses are typified by A/equine/Prague/56, which has H7 and N7, and A/equine/Hong Kong/92, which has H3 and N8 ([Table 23-1](#)).

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As influenza viruses spread through a population, they undergo mutation and selection and gradually change the structure of their hemagglutinins and neuraminidases. These changes gradually lead to alterations in the antigenicity of the virus. This gradual change is called antigenic drift, and it permits the virus to persist in a population for many years. In addition to antigenic drift, influenza viruses sporadically exhibit a sudden, major genetic change in which a new strain develops whose hemagglutinins show no apparent relationship to the hemagglutinins of previously known strains. Such a major change, called an antigenic shift, is not produced by mutation but results from recombination between two virus strains. The development of these influenza viruses with a completely new antigenic structure accounts for the periodic major outbreaks of influenza in humans and poultry. In horses and pigs, in contrast, the rapid turnover of the population and the constant production of large numbers of susceptible young animals ensures the persistence of influenza viruses without the necessity for extensive antigenic drift. As a result, the antigenic structure of equine and swine influenza viruses has changed only slowly since they were first described. Nevertheless, the H3N8 equine influenza virus strains are evolving into two distinct lineages, one European and one American, based on the structure of the HA1 domain of their hemagglutinin. Viruses of both lineages can circulate in horse populations at the same time. Examples of the European strains include A/Suffolk/89 and A/Hong Kong/1/92. American strains include A/Kentucky/94 and A/Florida/93. All are distinctly different from the original strain A/Miami/1/63.

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Table 23-1 Examples of Influenza A Viruses and Their Antigenic Structure

Species	Virus Strain	Antigenic Structure
Human	A/New Caledonia/20/99*	H1N1
	A/New Jersey/76 (swine flu)	H1N1
	A/Panama/2007/99	H3N2
Canine	A/Florida/04	H3N8
Equine	A/Equine/Prague/1/56	H7N7
	A/Equine/Miami/1/63	H3N8
	A/Equine/Suffolk/89	H3N8
	A/Equine/Hong Kong/1/92	H3N8
Swine	A/Swine/Iowa/15/30	H1N1
Avian	A/Fowl Plague/Dutch/27	H7N7
	A/Duck/England/56	H11N7
	A/Turkey/Ontario/6118/68	H8N4
	A/Chicken/Hong Kong/258/97	H5N1
	A/Chicken/Shantou/4231/2003	H5N1

* The first number is the isolate number; the second is the year of isolation.

A second form of immune evasion by viruses is seen in caprine arthritis-encephalitis (CAE), Aleutian disease of mink, and African swine fever. Although infected animals respond to these viruses, the antibodies formed are incapable of virus neutralization. Thus virus-antibody complexes from Aleutian disease-infected mink are fully infectious. Goats with CAE make large amounts of anti-envelope antibodies, but they develop negligible levels of neutralizing antibodies. In this case, goats fail to recognize and respond to the virus-neutralizing epitopes. If rabbits are immunized with CAE virus, they can readily produce virus-neutralizing antibodies and even goats will produce these antibodies if immunized with large amounts of an adjuvanted viral antigen. The antibodies produced in these hyperimmunized goats are very specific and will react only with the immunizing strain of the virus. Notwithstanding the absence of neutralizing antibodies, other antibodies can bind to CAE virions, and the opsonized virions are endocytosed by macrophages. Unfortunately, this virus grows within macrophages so that opsonizing antibodies merely speed up virus replication—an example of antibody-mediated enhancement. Attempts to vaccinate goats against CAE lead only to more severe disease.

A third mechanism by which viruses can evade destruction by antibodies is seen in yet another lentiviral infection, maedi-visna, a complex disease of sheep. (Maedi is a chronic pneumonia; visna is a chronic neurological disease caused by the same virus.) In maedi-visna infections, neutralizing antibodies are produced slowly. These neutralizing antibodies are unable to reduce the viral burden in infected sheep, and cyclical relapses do not occur. The antibodies have a low affinity for viral epitopes and take at least 20 minutes to bind to the virus and 30 minutes to neutralize it. In contrast, it takes only 2 minutes for this virus to infect a cell. Thus the virus can spread between cells much faster than it can be neutralized. The maedi-visna virus also invades monocytes and macrophages. In most of these cells the replication of the virus stops after its RNA has been

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reversely transcribed into proviral DNA. Cells are thus persistently infected by the virus without expressing viral antigens. The virus may therefore be disseminated without provoking immunological attack.

Maedi-visna is associated with extensive infiltration of the lungs, mammary gland, and central nervous system with MHC class II⁺ T cells (both CD4⁺ and CD8⁺) and macrophages. Immunosuppression reduces the severity of the lesions, whereas immunization against the virus increases their severity. It is suggested that persistently infected macrophages stimulate T cells to release cytokines. These cytokines delay the maturation of monocytes into macrophages and so restrict virus replication. They also enhance MHC class II expression on the macrophages and so trigger excessive T cell proliferation and chronic lymphoid hyperplasia.

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23.6.2

Interference with Interferons

Viruses use several methods to block the effectiveness of interferons. These range from blocking interferon receptor signal transduction to synthesizing soluble interferon receptors. Some viruses inhibit IFN- γ production by blocking the activities of IL-18 and IL-12, both of which are required for its production. For reasons unknown, some important viruses make chemokines or chemokine receptors. For example, equine herpesvirus makes CCR3, the receptor for CCL11. Marek's disease virus makes a protein related to CXCL8.

23.6.3

Inhibition of Cytotoxic T Cells and NK Cells

Viruses can inhibit T cell-mediated cytotoxicity by interfering with the expression of cell-surface MHC class I molecules. Thus bovine herpesvirus-1 downregulates the expression of MHC class I molecules by interfering with transporter protein functions. BHV-1 also downregulates the expression of mRNA for MHC class I molecules. Other viruses may cause MHC class I molecules to be retained within a cell; they may prevent peptide binding to transporter proteins; they may prevent proteasomal degradation; they may redirect MHC molecules to lysosomes for degradation; or they may encode inhibitors that block caspase activity. Adenoviruses possess proteins that block the activities of granzyme B. Some viruses can also block the killing activities of NK cells.

In contrast to the short-lived immune response against bacteria, antiviral immunity is, in many cases, very long-lasting. The reasons for this are unclear, but they are often related to virus persistence within cells, perhaps in a slowly replicating or a nonreplicating form as typified by the herpesviruses. It is usually difficult to isolate viruses from an animal that has recovered from a herpesvirus infection. Some time later, however, especially when the individual is stressed, the herpesvirus may reappear and may even cause disease again. During the latent period, when it is present in the host but cannot be reisolated, the virus nucleic acid persists in host cells but its transcription is blocked and viral proteins are not made. The persistent virus may periodically boost the immune response of the infected animal and in this way generate long-lasting immunity to superinfection. The immune responses in these cases, although unable to eliminate viruses, may prevent the development of clinical disease and therefore serve a protective role. Immunosuppression or stress may permit disease to occur in persistently infected animals. The association between stress and the development of some virus diseases is well recognized. It is likely that the increased levels of steroid production in stressful situations may be sufficiently immunosuppressive to permit activation of latent viruses or infection by exogenous ones.

Sometimes viruses may interact with bacteria to overcome the immune system. For example, it is well recognized that *Mannheimia haemolytica* and bovine herpesvirus-1 interact to cause severe respiratory disease in cattle. BHV-1 infection increases expression of the β 2-integrin CD11a/CD18 on lung neutrophils. The leukotoxin of *M. haemolytica* binds to this integrin and then kills the neutrophils, permitting growth of the invading bacteria.

23.7 VIRAL CYTOKINES

Some viruses produce proteins that are closely related to, or may affect, mammalian cytokines. These have been called virokines. Many of these virokines are suppressive molecules that inhibit antiviral immune responses. For example, cowpox virus makes an IL-1 β -binding protein that reduces the amount of IL-1 β available to promote an immune response. The human herpesvirus, Epstein-Barr virus, makes a protein closely related to IL-10 called vIL-10. Since IL-10 is an inhibitory cytokine, this effectively reduces T cell-mediated responses to this virus. Another example is the production of a protein related to the IFN- γ R by myxoma and poxviruses. Presumably, binding IFN- γ prevents the interferon from binding to cell receptors and inhibiting viral replication.

23.8 ADVERSE CONSEQUENCES OF IMMUNITY TO VIRUSES

The immune response to viruses can, on occasion, be a disadvantage. Indeed, there are many virus diseases in which the major lesions develop as a result of inappropriate or excessive immune responses. For example, bovine respiratory syncytial viruses induce a Th2 response in infected cattle with production of IL-4 and specific IgE antibodies in the lungs. This may result in a type I hypersensitivity reaction in the lungs since there is a direct correlation between lung IgE levels and the severity of clinical disease.

The destruction of virus-infected cells by antibody is classified as a type II hypersensitivity reaction (see [Chapter 26](#)), which, although normally beneficial,

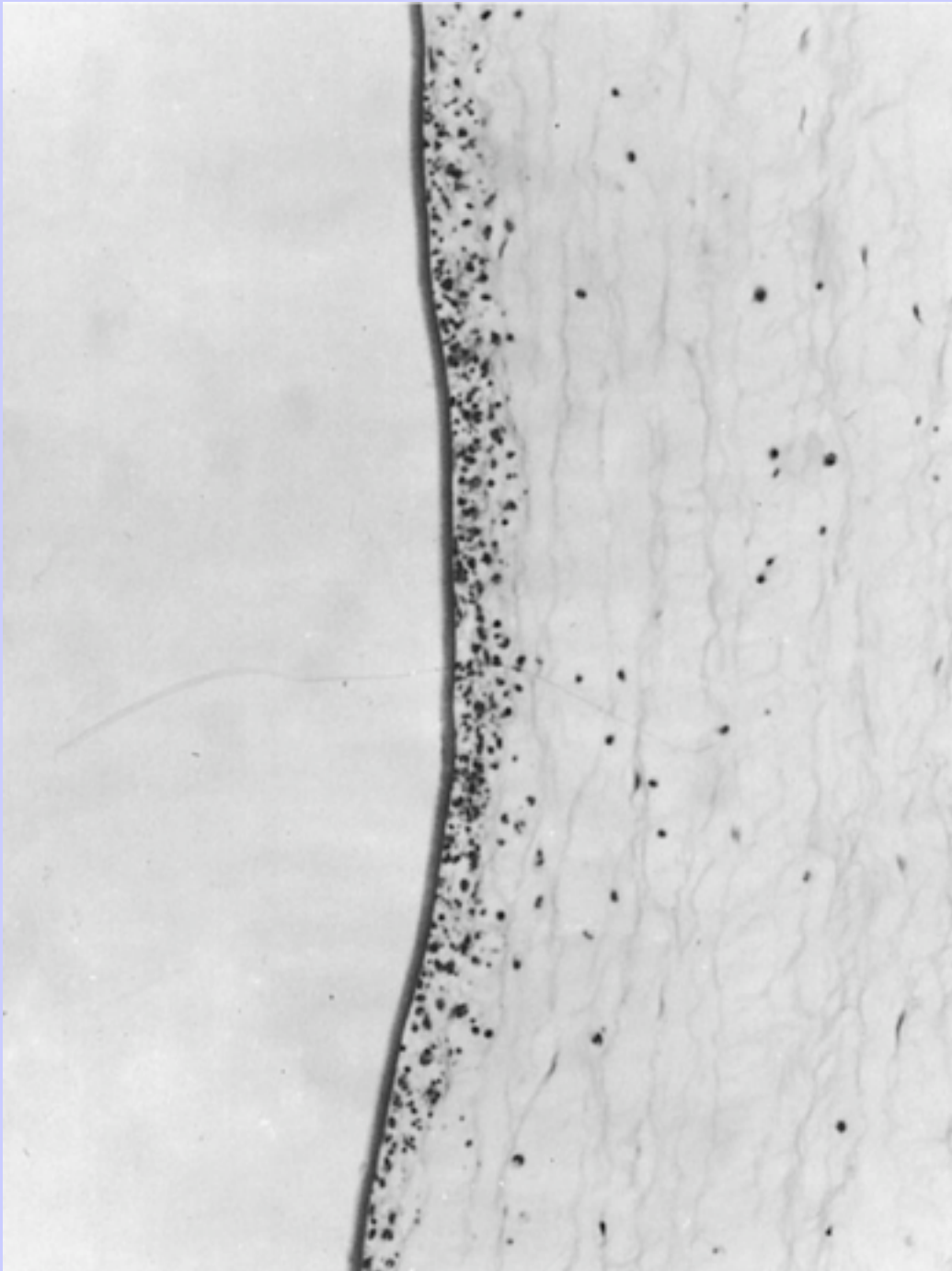
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FIGURE 23-7 A case of blue-eye in a Dachshund. This is a type III hypersensitivity reaction to canine adenovirus-1 (ICH) occurring in the cornea. (Courtesy Dr. H. Reed.)



FIGURE 23-8 A section from the cornea of a dog with blue-eye. Note the neutrophil infiltration of the posterior surface of the cornea as a result of virus-antibody complex deposition in this region. (From Carmichael LE: *Pathol Vet* 1:73-95, 1964.)



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may exacerbate virus diseases. Thus viruses are removed at the cost of cellular destruction. The severity and significance of this destruction depend on how widespread the infection becomes. In some diseases in which the virus causes little cell destruction, most of the tissue damage may result from immunological attack. A good example of this problem is seen in distemper encephalitis, in which neurons are demyelinated as a result of an antiviral immune response. Thus macrophages, which are numerous in these brain lesions, ingest immune complexes and infected cells. As a result they release oxidants and other toxic products. These toxic products damage nearby cells, especially oligodendroglia, and so cause demyelination. Old dog encephalitis, a disease of middle-aged dogs, is perhaps a variant of this postdistemper lesion.

It has been suggested that some cases of demyelinating encephalitis in canine distemper result from autoimmune attack. Thus most dogs with this syndrome make antibodies against myelin proteins, and it has been suggested that these cause tissue destruction. Their importance, however, is unclear. The level of antimyelin antibodies is unrelated to the severity of the disease, and in general, the animals with the highest titers are those that recover. On the other hand, some sera from affected dogs can cause demyelination in canine cerebellum cultures.

Type III (immune complex) lesions (see [Chapter 27](#)) are commonly associated with viral diseases, especially those in which viremia is prolonged. For example, a membranoproliferative glomerulonephritis resulting from the deposition of immune complexes is a common complication of EIA, Aleutian disease of mink, feline leukemia, chronic hog cholera, bovine viral diarrhea-mucosal disease, canine adenovirus infections, and FIP. A generalized vasculitis due to deposition of immune complexes throughout the vascular system is seen in EIA, Aleutian disease of mink, malignant catarrhal fever, and possibly, equine viral arteritis.

In dogs infected with canine adenovirus-1 (infectious canine hepatitis), an immune complex–derived uveitis and a focal glomerulonephritis both develop. The uveitis, commonly called “blue-eye,” is seen both in dogs with natural infections and in those vaccinated with live attenuated adenovirus vaccine ([Figure 23-7](#)). The uveitis results from the formation of virus-antibody complexes in the anterior chamber of the eye and in the cornea with complement activation and consequent neutrophil accumulation ([Figure 23-8](#)). The neutrophils release enzymes and oxidants that damage corneal epithelial cells, leading to edema and opacity. The condition resolves spontaneously in about 90% of affected dogs.

Finally, many virus diseases are associated with the occurrence of rashes. The pathology of these is complex but may reflect type II, III, or IV hypersensitivity reactions occurring as the host responds to the presence of viral antigens in the skin.

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23.8.1

Aleutian Disease of Mink

Although immune complex–mediated lesions are usually only of passing interest in many infectious diseases, they generate the major pathological lesions in Aleutian disease of mink. Aleutian disease is a persistent parvovirus infection that was first recognized in mink with the Aleutian coat color. Although all strains of mink are susceptible to this virus, Aleutian mink are genetically predisposed to the development of severe lesions since they are also affected by the Chédiak-Higashi syndrome (see [Chapter 34](#)). Persistently infected mink develop a slowly progressive lympho-proliferative disease with a plasmacytosis that has been compared to a myeloma, since it results in a polyclonal or monoclonal gammopathy ([Figures 23-9](#) and [23-10](#)). They also develop immune complex lesions (see [Chapter 27](#)), including glomerulonephritis and arteritis. They make autoantibodies to their own immunoglobulins (rheumatoid factors) and to DNA (antinuclear antibodies). Their serum IgG concentration increases, sometimes to very high levels. On occasion these elevated immunoglobulins are monoclonal in origin. They are directed against the Aleutian disease virus. The virus transforms B cells so that they proliferate and differentiate excessively.

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The immune complex-mediated lesions of Aleutian disease include an arteritis, in which IgG, C3, and viral antigen are found within vessel walls, and a glomerulonephritis, in which deposits of immune complexes containing virus and antibody are found. In addition, infected mink are anemic. Their red cells are coated with antiviral antibodies. It is likely, therefore, that the red cells of infected animals adsorb virus-antibody complexes from plasma. These coated red cells are then removed from the circulation by macrophages. As might be predicted, the use of immunosuppressive agents such as cyclophosphamide or azathioprine in infected mink prevents the development of many of these lesions and so prolongs survival, whereas experimental vaccination with inactivated Aleutian disease virus increases the severity of infections.

23.8.2

Feline Infectious Peritonitis

FIP is a fatal granulomatous disease of wild and domestic cats caused by a coronavirus. The FIP virus is closely related to feline enteric coronavirus, and viruses are found with properties intermediate between the two. However, feline enteric coronavirus prefers to replicate within intestinal epithelial cells, whereas FIP virus prefers to replicate within macrophages. Macrophages also spread the FIP virus throughout the body. FIP tends to infect relatively young cats between 6 months and 3 years of age. The disease presents in two major forms: an effusive

FIGURE 23-9 A section of liver from a mink infected with Aleutian disease. Note the marked plasma cell and mononuclear cell infiltration ($\times 250$). (From a specimen kindly provided by Dr. S.H. An.)

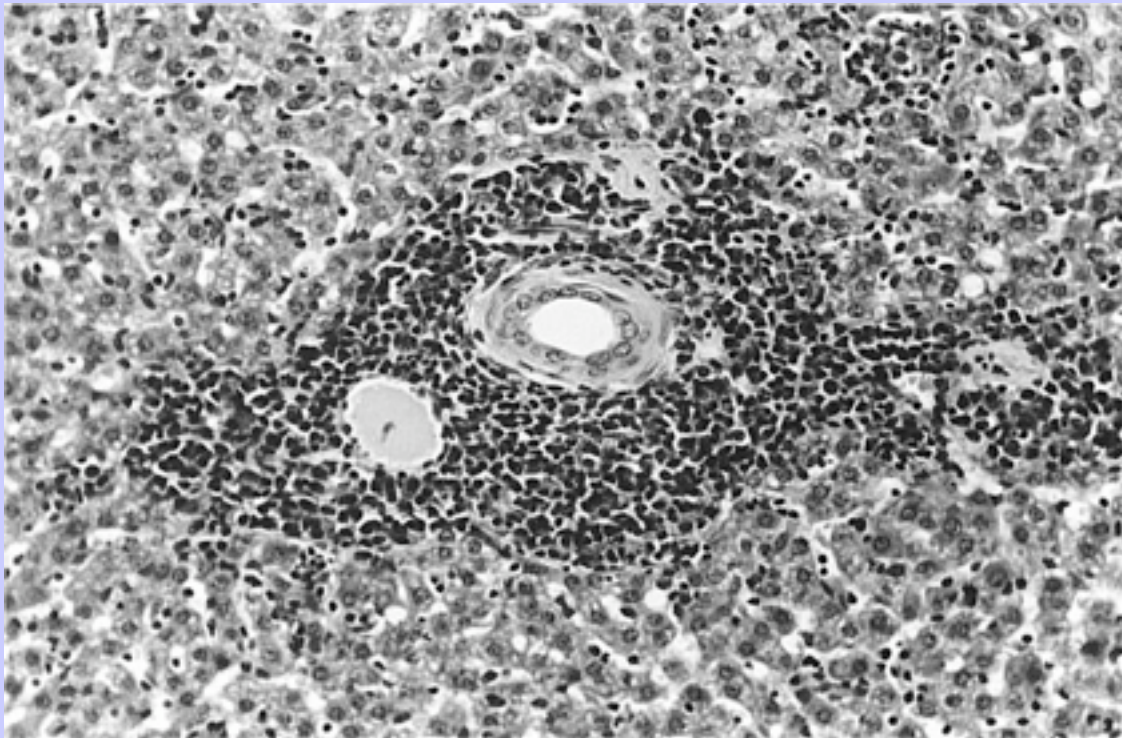
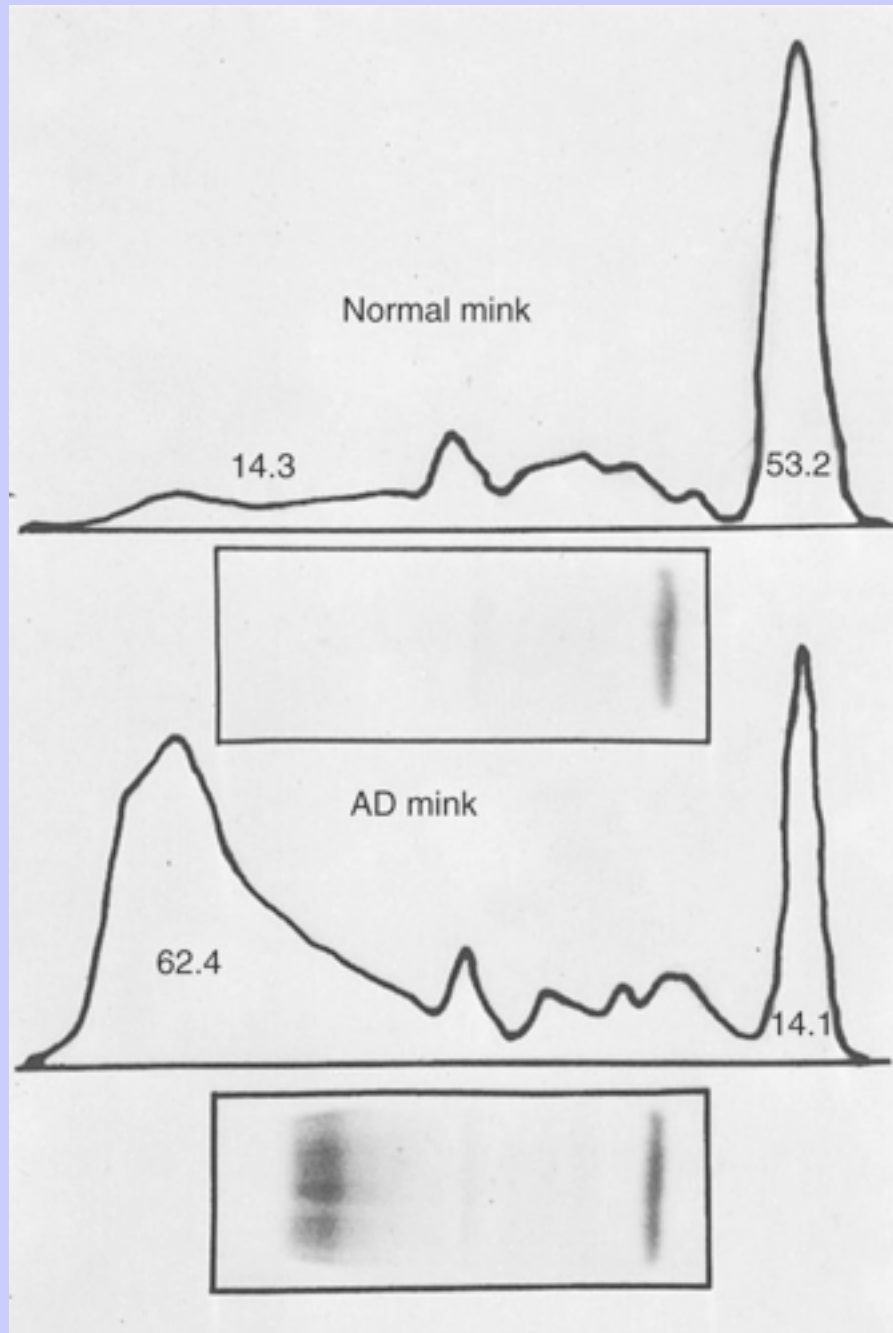


FIGURE 23-10 The serum protein electrophoretic patterns seen in normal and Aleutian disease (AD)-infected mink. The serum of the infected animal shows a polyclonal gammopathy so that the gamma globulins account for 62.4% of the serum proteins in contrast to the normal level of 14.3%. (Courtesy Dr. S.H. An.)



(“wet”) form with peritonitis (ascites) or pleuritis characterized by the presence of large amounts of proteinaceous fluid in the body cavities and associated with a vasculitis; and a noneffusive (“dry”) form characterized by multiple small granulomas on the surface of the major abdominal organs. Pleural lesions are uncommon in the noneffusive form of FIP. Some cats may show signs of central nervous system involvement and ocular lesions. Both forms of the disease are uniformly lethal, with affected cats dying between 1 week and 6 months.

The pathogenesis of FIP differs between the two forms of the disease. After invading a cat, the virus first replicates in intestinal epithelial cells. The virus shed by epithelial cells is then spread by monocytes and taken up by phagocytic cells in the target tissues. These target tissues include the serosa of the peritoneum and the pleura, as well as the meninges and the uveal tract. The course of the infection then depends on the nature of the immune response to the virus—a phenomenon also seen in several bacterial diseases (see [Chapter 22](#)). Immunity to FIP virus is entirely cellular, and it is likely that a Th1 response is protective. Antibodies, on the other hand, may exacerbate the disease. A cat that mounts a good Th1 response will become immune regardless of the amount of antibodies it makes. Some cats, however, probably make a Th2 response and fail to mount a cell-mediated immune response. In these animals, antibodies enhance virus uptake by macrophages in which the virus then replicates. Large numbers of virus-laden macrophages accumulate around the blood vessels of the omentum and serosa ([Figure 23-11](#)). These macrophages appear to be activated in that they are strongly positive for CD18 and produce TNF- α and IL-1 β . Endothelial cells upregulate MHC class II expression. These antibodies also generate immune complexes that are deposited in the serosa, causing pleuritis or peritonitis, and in glomeruli, leading to glomerulonephritis. The serosal vasculitis is responsible for the effusion of fibrin-rich fluid into the serosal cavities. This massive production of immune complexes may also be responsible for the disseminated intravascular coagulation seen in these cats. IL-1 and IL-6 are found in unusually high concentrations in the peritoneal fluid from cats with effusive FIP. Cats with preexisting high levels of antibodies against feline coronaviruses develop effusive FIP rapidly on challenge. Administering antiserum to feline coronavirus before FIP challenge may also accelerate the peritonitis. Compared to cats with FIP, coronavirus-infected cats without FIP express higher levels of IL-10 and macrophage colony-stimulating factor (M-CSF) in their spleen; higher levels of IL-12 p40 in their lymphoid tissues; lower levels of IL-1 β , IL-6, granulocyte colony-stimulating factor, and M-CSF; and higher levels of TNF- α in their mesenteric lymph nodes. It has been suggested that these coronavirus-infected cats do not develop FIP since they avoid excessive macrophage activation by upregulating IL-10.

A modified live intranasal vaccine is available against FIP. The vaccine contains a temperature-sensitive mutant virus that replicates in the upper respiratory tract and induces a local IgA response in the mucosa. This local mucosal response should prevent coronavirus invasion without inducing high levels of serum antibodies. This vaccine will, however, only be effective if administered before FIP exposure. In highly endemic situations in which kittens are infected at a young age, vaccination at 16 weeks of age may be too late to prevent infection.

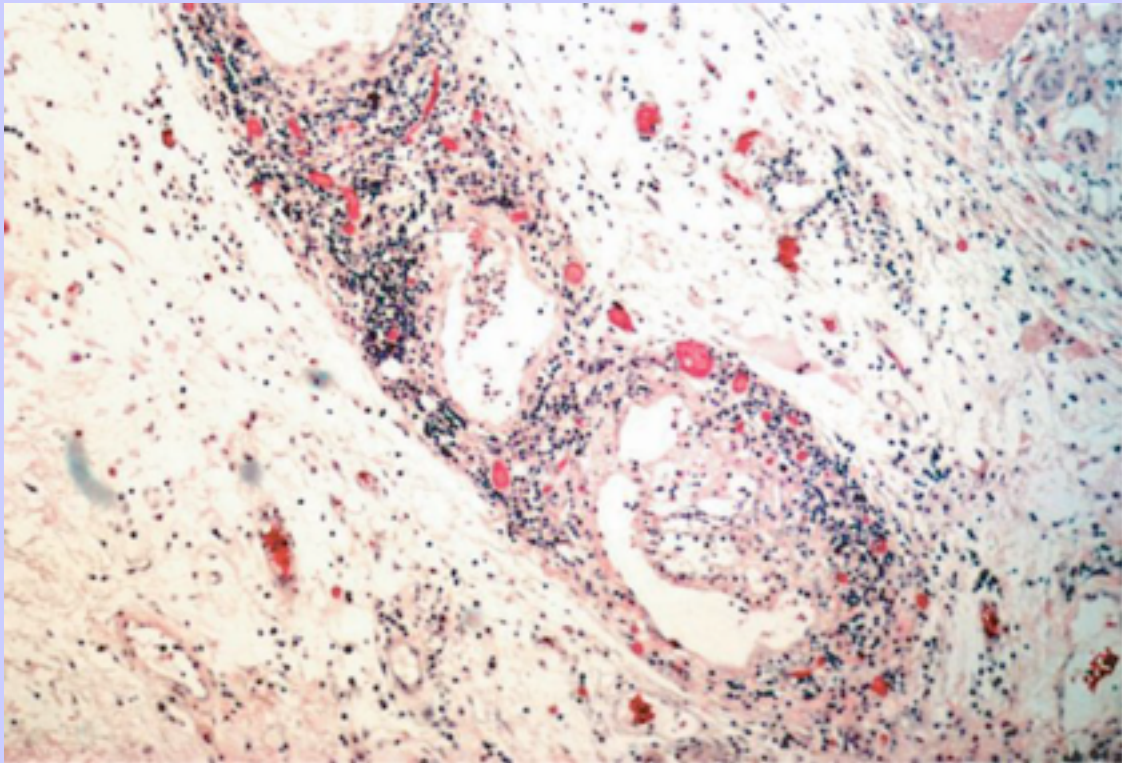
23.8.3 Equine Infectious Anemia

EIA is caused by a lentivirus. Following recovery from the first attack of clinical disease characterized by anemia, fever, thrombocytopenia, weight loss, and depression, horses may remain healthy for weeks or months. However, three or four relapses may occur before the horse either develops a chronic wasting disease or becomes clinically normal. The cyclical relapses may occur at 2- to 8-week intervals. Each episode of disease tends to be milder than the previous one. The fevers are lower and the anemia less severe. The EIA virus, like other lentiviruses, undergoes random mutation, and new, antigenically different variants are produced. The elimination of these variants is determined by the presence of neutralizing antibodies and cytotoxic T cells. As variant strains

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of the virus are produced, the infected horse makes neutralizing antibodies to that variant, and, as a result, the viremia ends. Variants of the EIA virus, however,

FIGURE 23-11 Granulomatous vasculitis of serosal blood vessels in a cat with feline infectious peritonitis. Note the marked cellular infiltration of the vessel adventitia and media. This reaction may be partially due to the deposition of virus-antibody complexes in the vessel walls. (Courtesy Dr. R.C. Weiss.)



appear rapidly and randomly. The appearance of a new nonneutralizable variant leads to a clinical relapse. After the virus has undergone several of these mutations and the horse has responded to them all, the neutralizing antibody spectrum of the horse's serum becomes very broad and viremia drops to a low level. Large amounts of tissues may then have to be examined to isolate the virus.

In addition to evading the immune response through antigenic variation, the EIA virus is associated with significant immunologically mediated tissue damage. The red cells of viremic horses adsorb circulating EIA virus onto their surface. Antibodies and complement then bind to the virus, as a result of which the red cells are cleared from the circulation more rapidly than normal. In addition to anemia, infected horses may also develop a membranoproliferative glomerulonephritis as a result of immune complex deposition on glomerular basement membranes. Horses infected with EIA have unusually low levels of IgG3, although their circulating lymphocytes appear to be unaffected and respond normally to mitogens such as phytohemagglutinin. The macrophage receptor for the EIA virus has been designated equine lentivirus receptor-1. It is a member of the family of TNF

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receptor proteins. It is possible that invasion of cells by the EIA virus is mediated by a single receptor in contrast to the paired co-receptors required by other lentiviruses.

23.8.4 Porcine Respiratory and Reproductive Syndrome

Porcine respiratory and reproductive syndrome (PRRS) is caused by a single-stranded, positive-sense RNA virus belonging to the family Arteriviridae. It infects pigs and causes a syndrome characterized by reproductive failure, infertility, abortions, anorexia, and secondary pneumonia. The virus replicates in macrophages and dendritic cells. (It kills M-DCs and suppresses the activities of P-DCs.) It shows a special preference for alveolar macrophages, whose destruction leads to an increase in secondary enzootic pneumonia. When the PRRS virus infects neonatal piglets, it causes a great increase in B cell numbers and activity. Thus they show polyclonal B cell activation, autoimmunity (antibodies specific for Golgi antigens and dsDNA), extreme lymphoid hyperplasia (grossly enlarged lymph nodes), and hypergammaglobulinemia (a 100- to 1000-fold increase in IgG; a 10- to 100-fold increase in IgM and IgA). The immunoglobulins produced are not directed against the PRRS virus, and the B cell proliferative response is not purely polyclonal. The antibodies produced are derived from a limited number of dominant B cell clones. It is speculated that the virus produces some form of B cell superantigen. Affected pigs also show a relative decrease in CD4⁺ T cells and an increase in CD8⁺ cells after several weeks. Cell-mediated responses and virus-neutralizing antibodies to the PRRS virus do not develop for about four weeks as a result of the loss of CD4 cells. Because of this immunosuppression, the PRRS virus may cause persistent infections that may take as long as 6 months to clear.

23.9 SEROLOGY OF VIRAL DISEASES

23.9.1 Tests to Detect and Identify Viruses

Among the simplest and most widely employed tests for the detection of viruses are the direct and indirect fluorescent antibody tests used to identify virus in the tissues of infected animals (see [Chapter 38](#)). If these methods are not possible, it may be necessary to grow the virus in experimental animals, chick embryos, or tissue cultures to provide sufficient antigen for testing. Once sufficient virus has accumulated, it can be identified by its reaction with specific antiserum. The tests commonly employed for this purpose are fluorescent antibody tests, enzyme-linked immunosorbent assay (ELISA), hemagglutination inhibition, virus neutralization, complement fixation, and gel precipitation. The precise tests employed will depend on the nature of the unknown virus. Hemagglutination inhibition tests are technically simple and are preferred if available. They do, however, tend to be strain-specific. The complement fixation test and gel precipitation test, as a broad generalization, tend to be largely group-specific and thus lend themselves to attempts to identify the genus to which a virus belongs. In contrast, virus neutralization tests tend to be highly strain-specific, so much so that they are perhaps best employed in the classification of a virus into its subtypes rather than in identifying the specific genus of a particular organism. There are, of course, exceptions to these generalizations; for example, the New Jersey and Indiana strains of vesicular stomatitis virus do not cross-react in the complement fixation test.

A popular technique for the detection of viral antigen or antiviral antibodies is the membrane filter ELISA test (see [Chapter 38](#)). This test has the advantage that both positive and negative controls can be incorporated with the test serum in one well. In addition to serum, whole blood, plasma, or saliva may be employed as a source of antigen or antibody.

Specific antibodies can be used to enrich virus suspensions before electron microscopy. For example, a fecal sample may be centrifuged, leaving a clear supernatant that contains a small number of many different viruses.

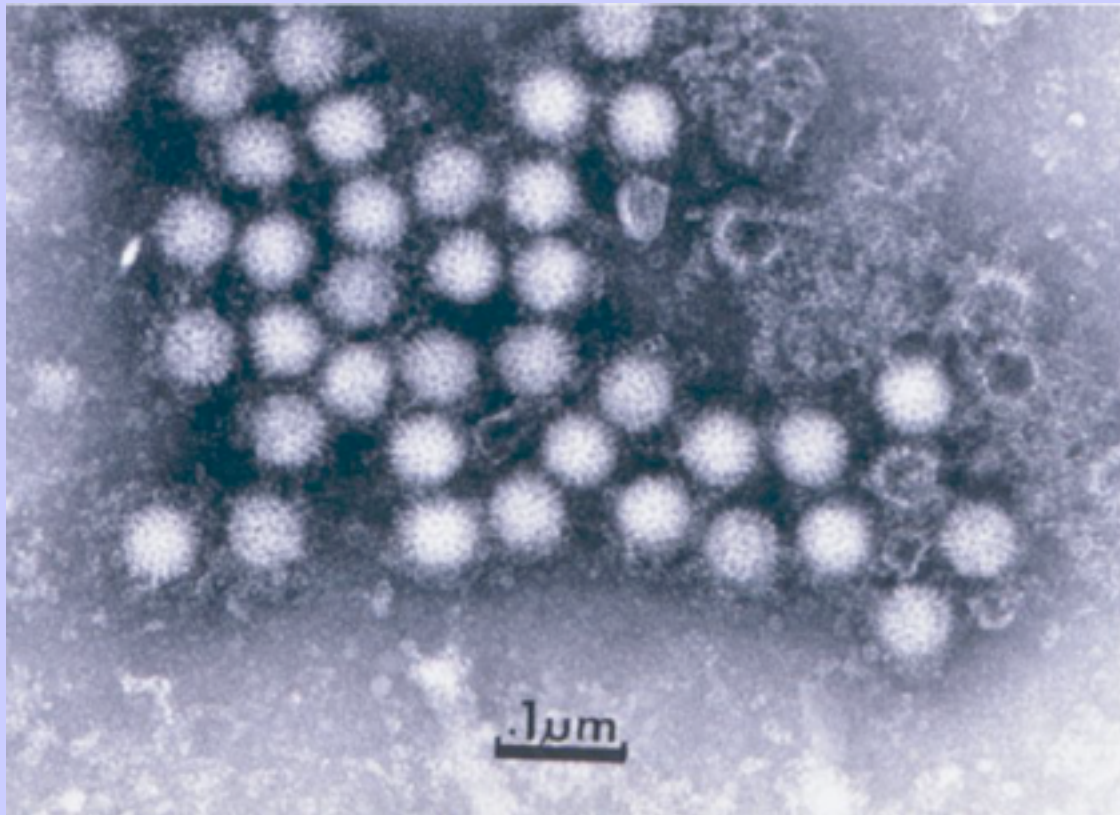
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After sonication to break up clumps, antibody specific for the virus of interest is added to the supernatant, and after a brief incubation, the fluid is centrifuged

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FIGURE 23-12 Immunolectron microscopy of porcine rotavirus clumped by convalescent antiserum ($\times 130,500$). (Courtesy Dr. L. Saif.)



again. Virus particles clumped by antibody are spun to the bottom, where they can be removed and examined by electron microscopy after negative staining ([Figure 23-12](#)). The antibody, by clumping only the virus of interest, renders it much more visible in the electron microscope, and the presence of visible antibody within the virus clumps provides direct confirmation of the identity of the virus.

23.9.2 Tests to Detect and Identify Antiviral Antibodies

In general, the most widely employed techniques for detecting antibodies to viruses are hemagglutination inhibition, indirect ELISA, immunofluorescence, gel diffusion, Western blotting, complement fixation, and virus neutralization. The first four of these are technically simple and are thus preferred. The complement fixation test and the virus neutralization tests are complex, thus restricting the circumstances in which they may be used. The virus neutralization tests are also extremely specific, which, as discussed earlier, tends to reduce their value as screening tests.

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24 CHAPTER 24 Acquired Immunity to Parasites

24.1 KEY POINTS

- Parasites by definition are able to evade their host's immune response for at least sufficient time for the parasite to reproduce.
- In general antibody-mediated immune responses protect against extracellular protozoa, whereas cell-mediated responses control intracellular protozoa.
- Protozoan parasites employ some very sophisticated techniques to ensure their survival in the face of an animal's immune response.
- Helminth parasites have a unique ability to trigger Th2 responses and immunoglobulin E (IgE) production. IgE may have evolved as an antiparasite antibody.
- Parasitic worms have a thick cuticle that protects them against damage caused by most protective cells. However, eosinophils appear to be uniquely able to damage and kill helminths.
- Immunity to parasitic arthropods such as ticks and biting flies also appears to be a property of the Th2 responses. However, although immune responses can reduce arthropod feeding and reproduction, they rarely kill them.

Infectious disease is, as pointed out earlier, rarely a result of the activities of a malicious microorganism. In the vast majority of cases, disease occurs because of the host's reaction to the infection or because the invader inadvertently causes significant damage to its host. Well-adapted parasites do not make these mistakes. They have evolved in such a way that their presence in the host is scarcely noticed. They exploit the host's resources without causing irreparable damage or triggering an effective defensive response. Thus parasitic infections caused by protozoan parasites or helminths may be noticed only by production losses. Indeed, in many cases, the presence of parasites comes to our attention only when they are present in unusually large numbers or when they damage a critical organ by accident. Sometimes, of course, a parasite may deliberately cause disease. For instance, parasites such as *Toxoplasma*, transmitted by carnivorism, may benefit by causing their host to become slow or confused so that it may be more readily eaten by a predator.

The consistent feature of all parasite infestations, however, is that they block or significantly delay the innate and acquired defenses of their host so that they may persist for sufficient time to reproduce. Some parasites may simply delay their destruction until they complete a single life cycle. Other well-adapted parasites may contrive to survive for the life of their host, protected from immunological attack by sophisticated and specific evasive strategies.

In contrast to the acute, short-lived infections caused by bacteria and viruses, infections by parasitic protozoa or helminths are long-lasting. Ideally, a successful parasite will regulate a host's immune responses, suppressing these to permit parasite survival, while at the same time allowing other responses to proceed and so preventing the death of the host from other infections. In addition, many parasites make use of the host's metabolic or control pathways for their own purposes. Thus epithelial growth factor and interferon- γ (IFN- γ) can enhance the growth of *Trypanosoma brucei*, and interleukin-2 (IL-2) and granulocyte-macrophage colony-stimulating factor can promote the growth of *Leishmania amazonensis*. The sharing of cytokines by host and parasites in this way reflects the long history of their association and their success in adapting to a parasitic lifestyle. It is evident that these parasites must have evolved very effective mechanisms to prevent immunological destruction.

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24.2 IMMUNITY TO PROTOZOA

24.2.1 Innate Immunity

The mechanisms of innate resistance to protozoa appear to be similar to those that prevent bacterial and viral invasion. Species influences appear to be of much greater significance. For example, *T. brucei*, *Trypanosoma congolense*, and *Trypanosoma vivax* do not cause disease in the wild ungulates of East Africa but will kill domestic cattle, presumably as a result of lack of mutual adaptation. Similarly, the coccidia are extremely host-specific: for example, *Toxoplasma gondii* tachyzoites can infect any species of mammal, but its coccidian stages affect only felids (e.g., cats, tigers).

Presumably, these species differences are but a reflection of more subtle genetic influences. Thus some breeds of African cattle, most notably N'Dama, show increased resistance to infection by pathogenic trypanosomes. This trypanotolerance results from natural selection of the most resistant animals over many generations and reflects their greater ability to control infection, as well as their resistance to the pathological effects of the parasite. Thus the γ/δ T cells of N'Dama are much more responsive to trypanosome antigens than are the γ/δ T cells of nonnative cattle. Trypanotolerant animals produce more IL-4 and less IL-6 than susceptible animals. At the same time, trypanotolerant animals show neither the severe anemia nor the production loss seen in susceptible cattle. Trypanotolerant animals produce high levels of immunoglobulin G (IgG) against *T. congolense* cysteine protease. Since this enzyme contributes to the pathology of infection, these antibodies may partially account for trypanotolerance.

Perhaps the best-analyzed example of genetically determined resistance to a protozoan infection is sickle cell anemia and its role in resistance to malaria in humans. Individuals who inherit the sickle cell trait possess hemoglobin S (HbS), in which a residue of valine has replaced a residue of glutamic acid present in normal hemoglobin. This alteration causes deoxygenated HbS molecules to precipitate when reduced, thus distorting the shape of the erythrocytes and resulting in increased erythrocyte fragility and clearance. Individuals who are homozygous for the sickle cell gene die from severe anemia when young. Heterozygous individuals are also anemic, but in west central Africa the fact that HbS kills *Plasmodium falciparum* ensures that affected individuals are resistant to malaria. As a result of this, more of these individuals tend to survive to reproductive age than do normal persons. The mutation is therefore maintained in the human population at a relatively high level.

24.2.2 Acquired Immunity

Like other invaders, protozoa stimulate both antibody- and cell-mediated immune responses. In general, antibodies control the numbers of parasites in blood and tissue fluids, whereas cell-mediated responses are directed largely against intracellular parasites.

Serum antibodies directed against protozoan surface antigens may opsonize, agglutinate, or immobilize them. Antibodies together with complement and cytotoxic cells may kill them, and some antibodies (called ablastins) may inhibit their division. In genital infections of humans due to *Trichomonas vaginalis*, a local IgE response is stimulated. The allergic reaction that ensues provokes intense discomfort; more important, by increasing vascular permeability, this reaction permits IgG antibodies to reach the site of infection and immobilize and eliminate the organisms.

In babesiosis the infective stages of the organisms (sporozoites) invade red blood cells. Infected red cells incorporate *Babesia* antigens into their membranes. These in turn induce antibodies that opsonize the red cells and cause their removal by phagocytosis. Infected red cells may also be destroyed by antibody-dependent cell-mediated responses. Macrophages and cytotoxic lymphocytes can recognize the *Babesia* antigen/opsonizing-antibody complex on the surface of infected erythrocytes. Cytotoxic T cells may play a role early in infection, when the number of infected erythrocytes is small.

Intracellular parasites use many different and unique strategies to invade cells and inhibit intracellular killing. Most gain entry to a cell by employing host-mediated processes such as phagocytosis or induced uptake.

Apicomplexans such as *Toxoplasma* and *Cryptosporidium*, however, actively penetrate cells using a system of adhesion-based motility called “gliding.” Once inside, these parasites reside in specially modified vacuoles.

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Protective immunity against apicomplexan protozoa such as *Cryptosporidia*, *Eimeria*, *Neospora*, *Plasmodia*, and *Toxoplasma* is generally mediated by Th1 responses. For example, *T. gondii* is an obligate intracellular parasite whose tachyzoites grow within cells ([Figure 24-1](#)). Eventually, the infected cell ruptures and the tachyzoites are released to invade other cells. They penetrate these cells by “gliding” through a molecular junction in the cell membrane and so do not trigger proper phagosome formation and maturation. *Toxoplasma* tachyzoites are therefore not destroyed, since their “parasitophorous vacuoles” do not mature and fuse with lysosomes. As a result, *Toxoplasma* can grow inside cells in an environment free of antibodies, oxidants, or lysosomal enzymes.

Normally, both Th1 and Th2 immune responses are triggered by *Toxoplasma*. The Th2 response generates antibodies that, together with complement, destroy extracellular organisms and prevent its spread between cells ([Figure 24-2](#)). This response, however, has little or no influence on the intracellular forms of the parasite. The intracellular organisms are destroyed by an IL-12–dependent Th1 cell–mediated response. Sensitized Th1 cells secrete IFN- γ in response to *Toxoplasma* ribonucleoproteins. This IFN- γ activates macrophages, enabling them to kill the intracellular organism by permitting lysosome-phagosome fusion. Some T cells may also secrete cytokines that interfere directly with *Toxoplasma* replication. In addition, cytotoxic T cells can destroy *Toxoplasma* tachyzoites and *Toxoplasma*-infected cells. Thus both Th1 and Th2 immune responses act together to ensure the elimination of the tachyzoites. However, *T. gondii* tachyzoites can develop into a cyst form containing bradyzoites. The cysts are weakly immunogenic and do not stimulate inflammation. It is possible that this cyst stage is not recognized as foreign.

Th1-mediated responses involving activation of macrophages are important in many protozoan diseases where the organisms are resistant to intracellular destruction. One of the most significant destructive pathways in M1 cells is the production of nitric oxide. Nitrogen radicals formed by the interaction of NO with oxidants are lethal for many intracellular protozoa.

FIGURE 24-1 Mouse macrophages containing healthy, growing tachyzoites of *Toxoplasma gondii*. Once an immune response develops, these cells become activated and acquire the ability to destroy ingested tachyzoites. (Courtesy Dr. C.H. Lai.)

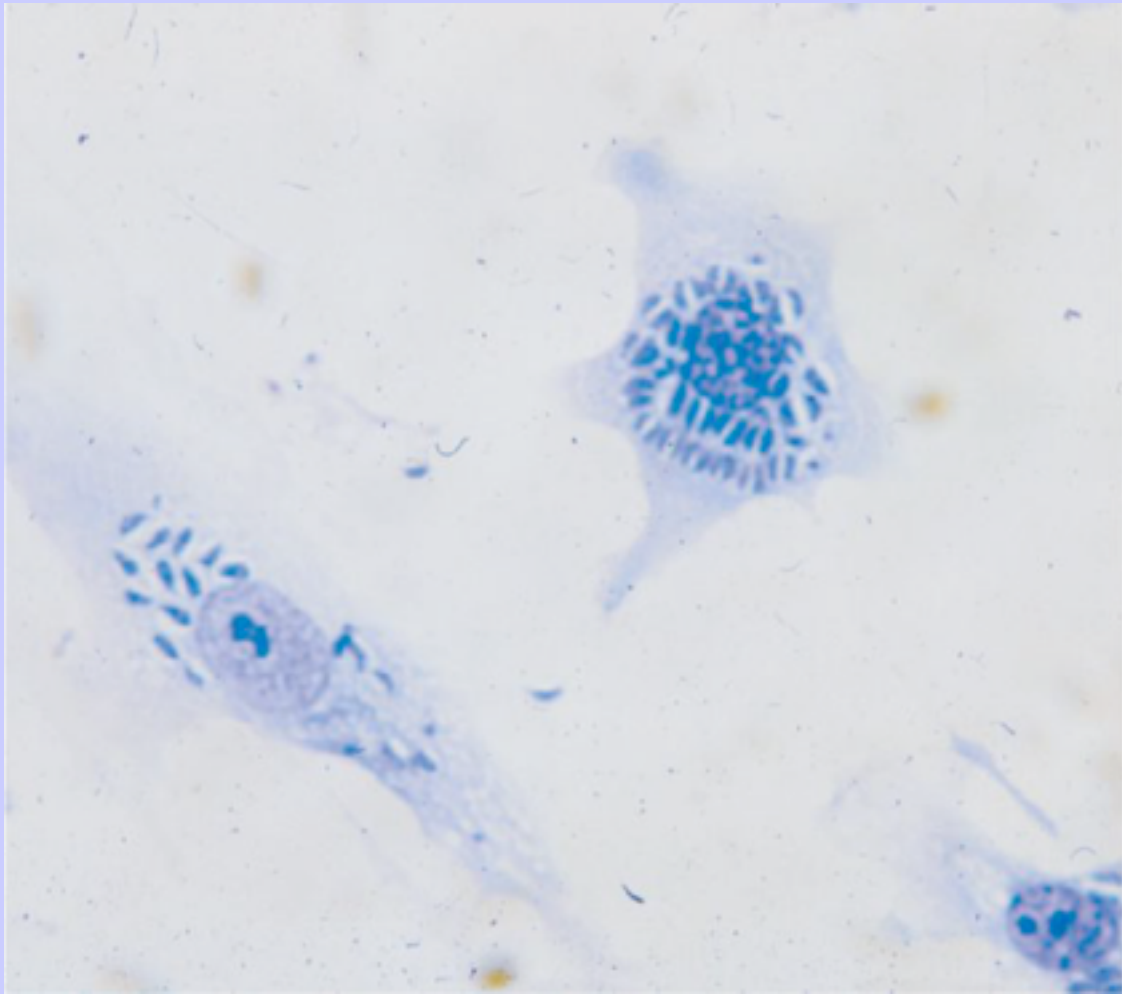
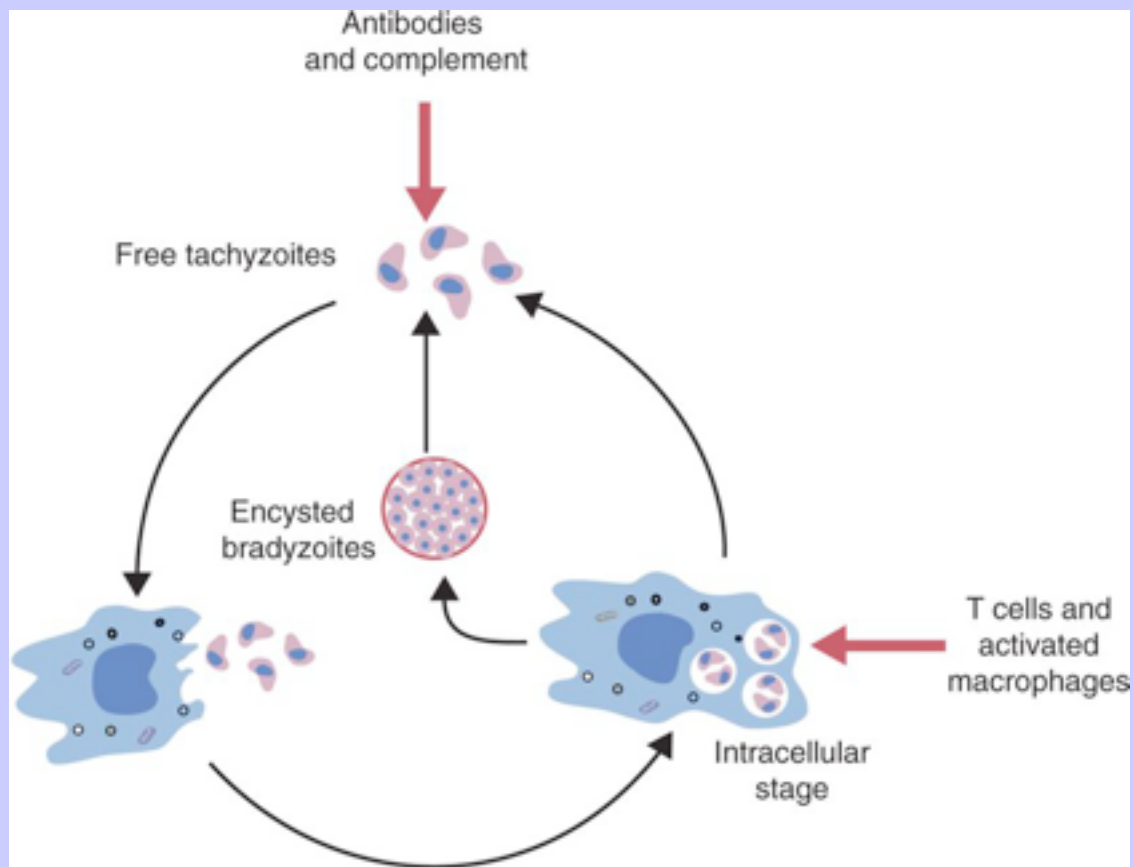


FIGURE 24-2 The points in the life cycle of *Toxoplasma gondii* at which the immune system can exert a controlling influence.



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However, protozoa are also experts in surviving within macrophages. For example, *Leishmania*, *Toxoplasma*, and *Trypanosoma cruzi* can migrate into safe intracellular compartments by stalling phagosome maturation. *Leishmania* and *T. cruzi* can suppress the production of oxidants or cytokine production, whereas *T. gondii* can promote macrophage apoptosis. *T. gondii* tachyzoites inhibit proinflammatory cytokine production by preventing nuclear translocation of nuclear factor kappa-B (NF- κ B).

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In *Theileria parva* infection (East Coast fever) of cattle, sporozoites preferentially invade lymphocytes and induce uncontrolled cell proliferation. *T. parva* can invade α/β and γ/δ T cells, as well as B cells. The parasite then activates NF- κ B by continuously phosphorylating its inhibitor proteins I κ -Ba and I κ -Bb (see [Chapter 36](#)). NF- κ B thus persists, maintains the cell in an activated state, and prevents its apoptosis. The activated cells produce both IL-2 and IL-2R. As a result, a loop is established, by which infected cells secrete IL-2, which in turn stimulates their growth. As *Theileria* shizonts develop within lymphocytes, the infected cells enlarge and proliferate. Since the parasite divides synchronously with its host cell, there is a rapid increase in parasitized cells. In most cattle this results in overwhelming infection and death. Some animals, however, recover from infection and become solidly immune. In these animals, CD8⁺ T cells can kill infected lymphocytes by recognizing parasite antigens in association with major histocompatibility complex (MHC) class I molecules. In susceptible animals, the parasites interfere with MHC class I expression.

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Infection of chickens or mammals with *Eimeria* oocysts generally leads to strong, species-specific immunity that can prevent reinfection. This immune response inhibits the growth of trophozoites, the earliest invasive stage, within intestinal epithelial cells. This growth inhibition is reversible, since arrested stages can be transferred to normal animals and complete their development uneventfully. Studies in mice suggest that resistance to primary infection is mediated by multiple cell-mediated mechanisms that include CD4⁺ T cells and their cytokines IL-12 and IFN- γ , macrophages, and natural killer (NK) cells. In contrast, resistance to secondary challenge is mediated by CD8⁺ T cells. In chickens, IFN- γ , tumor necrosis factor- α (TNF- α), and transforming growth factor- β , as well as intraepithelial CD8⁺ α/β T cells, appear to be essential in host immunity. It is interesting to note that *Eimeria*-susceptible chickens express much more IL-10 in their intestines than do resistant chickens, both constitutively and after infection. Given that IL-10 promotes a Th2 bias, it is likely that it effectively reduces immunity to this parasite.

For many years it was thought that a common feature of many protozoan infections was *premunity*, a term used to describe resistance that is established after the primary infection has become chronic and is only effective if the parasite persists in the host. It was believed, for example, that only cattle actually infected with *Babesia* were resistant to clinical disease. If all organisms were removed from an animal, resistance was believed to wane immediately. Studies have shown that this is not entirely true. For example, cattle cured of *Babesia* infection by chemotherapy are resistant to challenge with the homologous strain of that organism for several years. Nevertheless, the presence of infection does appear to be mandatory for protection against heterologous strains. Babesiosis is also of interest since splenectomy of infected animals will result in clinical disease. The spleen not only serves as a source of antibodies in this disease but also removes infected erythrocytes. Loss of these functions permits the clinical disease to reappear.

24.2.2.1

Leishmaniasis

The importance of specific immune responses in determining the course and nature of a protozoan disease is best seen in canine leishmaniasis. Leishmaniasis is caused by protozoan parasites of the genus *Leishmania* and transmitted by biting sandflies. When the promastigote forms of this parasite are injected into dogs percutaneously by its sandfly vector, they are rapidly phagocytosed by macrophages. *Leishmania* parasites are obligate intracellular pathogens. Thus they divide within the macrophages until the cells rupture and the released organisms are then phagocytosed by neighboring cells. Depending upon the degree of immunity in the host, the parasites may be restricted to the skin (cutaneous disease) or infected macrophages may enter the circulation and lodge in the internal organs, leading to disseminated visceral disease. Most dogs are resistant: Only 10% of infected dogs develop clinical disease.

Surviving parasites divide within the phagolysosomes of infected macrophages. Their resistance to intracellular destruction is a result of multiple mechanisms. *Leishmania* lipophosphoglycan delays phagosome maturation, thus preventing the production of nitric oxide and inhibiting many of the responses of macrophages to cytokines. (One study of 245 macrophage genes showed that 37% were suppressed by *Leishmania* infection.) The parasite also suppresses the antigen-presenting ability of macrophages by suppressing MHC class II expression. As a result of their persistence, the parasites trigger chronic inflammation. Initially characterized by the presence of granulocytes, this is followed by macrophages, lymphocytes, and NK cells, which collectively form granulomas.

The clinical signs of *Leishmaniasis* are directly linked to the immune response mounted by the infected animal. In susceptible animals, the organisms may spread from the skin to the local lymph node, spleen, and bone marrow within a few hours. In resistant dogs, the parasites remain restricted to the skin and draining

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lymph node. As a result, the dogs either remain healthy or develop a mild, self-limiting disease. These resistant dogs characteristically mount a Th1 response to the parasites. They mount a weak antibody response but a strong and effective cell-mediated one. Thus while they may have low antibody titers, they produce IFN- γ in response to parasite antigens, have large numbers of T cells in their granulomas, mount strong delayed hypersensitivity responses, and eventually demonstrate very effective intracellular destruction of the parasites. Destruction of intracellular parasites results from activation of their macrophages by T cell-derived IFN- γ . Resistance to *Leishmania* has a strong genetic component: for example, Ibizian Hounds appear to be resistant to this parasite. There is an association between resistance and certain MHC class II haplotypes as well as certain Nrap alleles in dogs (see [Chapter 4, Box 4-1](#)).

Susceptible dogs, in contrast, mount a Th2 response characterized by high antibody levels but poor cell-mediated immunity. The differences have been attributed to the activities of IL-10-producing T_{reg} cells. In addition, the parasite may actively suppress transcription of the IL-12 gene and so ensure that a Th2 response predominates. Because this is an intracellular parasite, a chronic, progressive disease results in these susceptible dogs. Parasite-laden macrophages accumulate, but the organism continues to multiply. These macrophages spread throughout the body, resulting in disseminated infection. Dogs develop severe generalized nodular dermatitis, granulomatous lymphadenitis, splenomegaly, and hepatomegaly. These dogs show polyclonal (occasionally monoclonal) B cell activation involving all four IgG classes and hypergammaglobulinemia and develop lesions associated with type II and type III hypersensitivity. This excessive immunoglobulin production can lead to development of an immune-mediated hemolytic anemia, thrombocytopenia, and the appearance of antinuclear antibodies. Glomerulonephritis, uveitis, and synovitis may result from chronic immune complex deposition leading to renal failure and death.

24.2.3

Evasion of the Immune Response

Despite their antigenicity, parasitic protozoa manage to survive within their host by using multiple evasion mechanisms that have been acquired over many millions of years of evolution. For example, *T. gondii* can avoid neutrophil attachment and phagocytosis. *T. parva* invades and destroys T cells. Other protozoa such as the trypanosomes may promote the development of suppressive regulatory cells or stimulate the B cell system to exhaustion. *P. falciparum* can suppress the ability of dendritic cells to process antigen.

Parasite-induced immunosuppression may promote parasite survival. For example, *Babesia bovis* is immunosuppressive for cattle. As a result, its host vector, the tick *Boophilus microplus*, is better able to survive on infected animals. Thus infected cattle have more ticks than noninfected animals, and the efficiency of transmission of *B. bovis* is enhanced. It must be pointed out, however, that parasite-induced immunosuppression can kill the host as a result of secondary infection, so it is not always beneficial to the parasite. Death in bovine trypanosomiasis is commonly due to bacterial pneumonia or sepsis following immunosuppression.

In addition to immunosuppression, protozoa have evolved two other effective evasive techniques. One involves becoming nonantigenic, and the other involves the ability to alter surface antigens rapidly and repeatedly. An example of a nonantigenic organism is the cyst stage of *T. gondii*, which, as mentioned previously, does not appear to stimulate a host response. Some protozoa can become functionally nonantigenic by masking themselves with host antigens. Examples of these include *Trypanosoma theileri* in cattle and *Trypanosoma lewisi* in rats, both nonpathogenic trypanosomes that survive in the blood of infected animals because they become covered with a layer of host serum proteins and so are not regarded as foreign. *T. brucei*, a pathogenic trypanosome of cattle, may also adsorb host serum proteins or soluble red cell antigens and so reduce its antigenicity.

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Although the absence of antigenicity may be considered the ultimate evasive technique, many protozoa, especially the trypanosomes, successfully employ repeated antigenic variation. If cattle are infected with the pathogenic trypanosomes *T. vivax*, *T. congolense*, or *T. brucei* and their parasitemia is measured at regular intervals, the numbers of circulating organisms are found to fluctuate greatly. Periods of high parasitemia alternate regularly with periods of low or undetectable parasitemia (Figure 24-3). Serum from infected animals contains antibodies against trypanosomes isolated before bleeding but not against those that develop subsequently. Each period of high parasitemia corresponds to the expansion of a population of trypanosomes with a new surface glycoprotein antigen. The elimination of this population by antibodies leads to a rapid fall in parasitemia. Among the survivors, however, some parasites express new surface glycoproteins and grow without hindrance. As a result, a fresh population arises to produce yet another period of high parasitemia (Figure 24-4). This cyclical

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FIGURE 24-3 The time course of *Trypanosoma congolense* parasitemia in an infected calf. Each parasitemic peak represents the development of a new, antigenically original population of organisms.

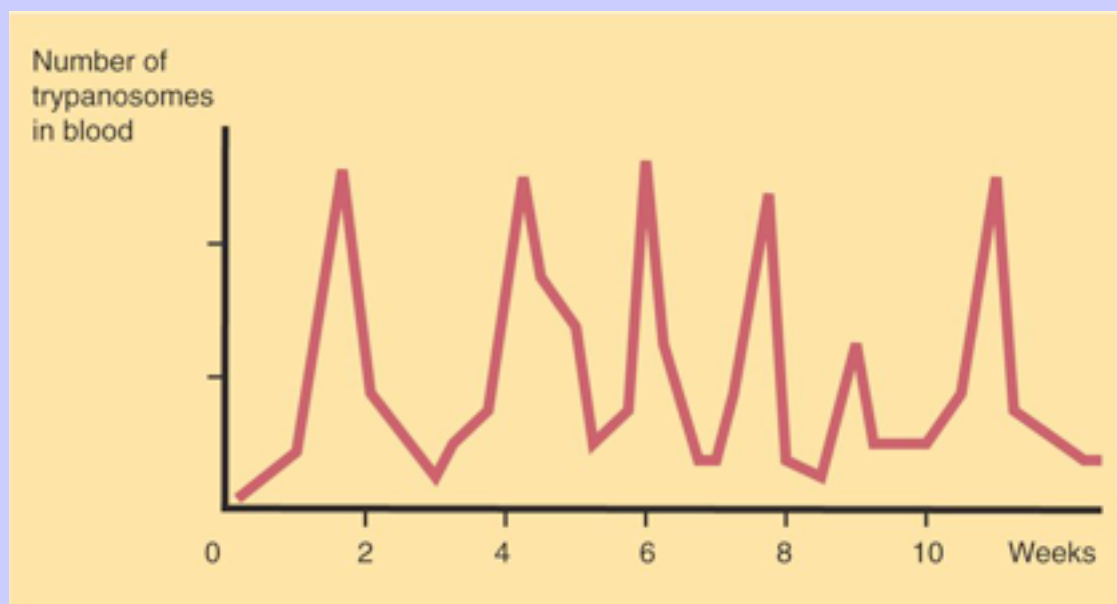
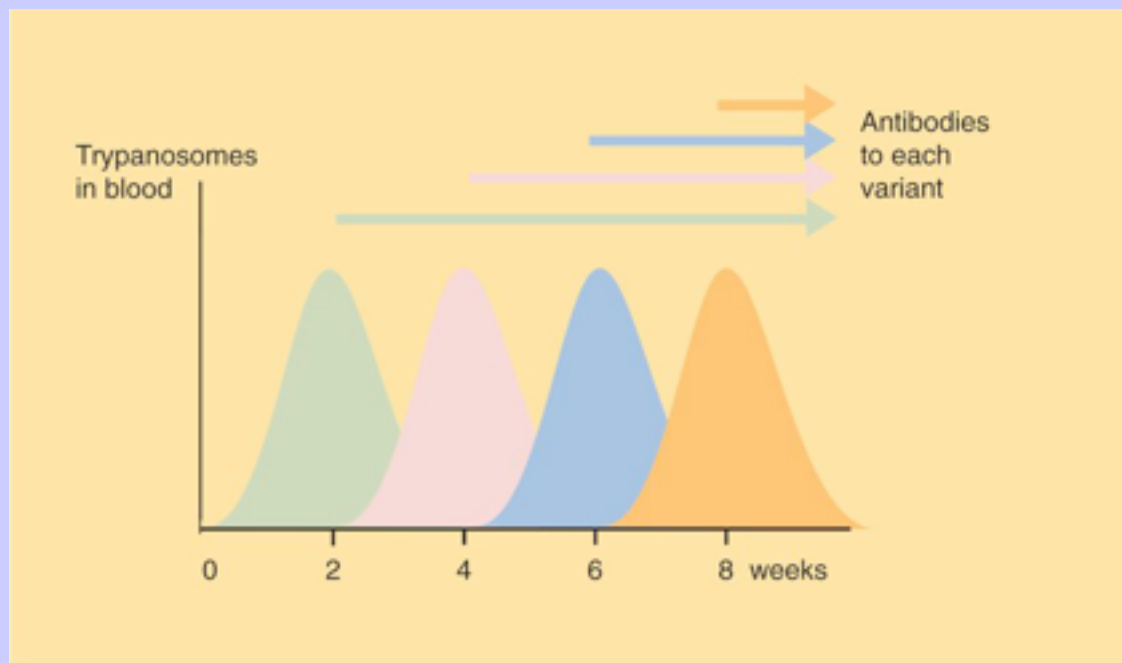


FIGURE 24-4 A schematic diagram showing how repeated antigenic variation accounts for the cyclical parasitemia observed in African trypanosomiasis. Each peak represents the growth of a new antigenic variant.

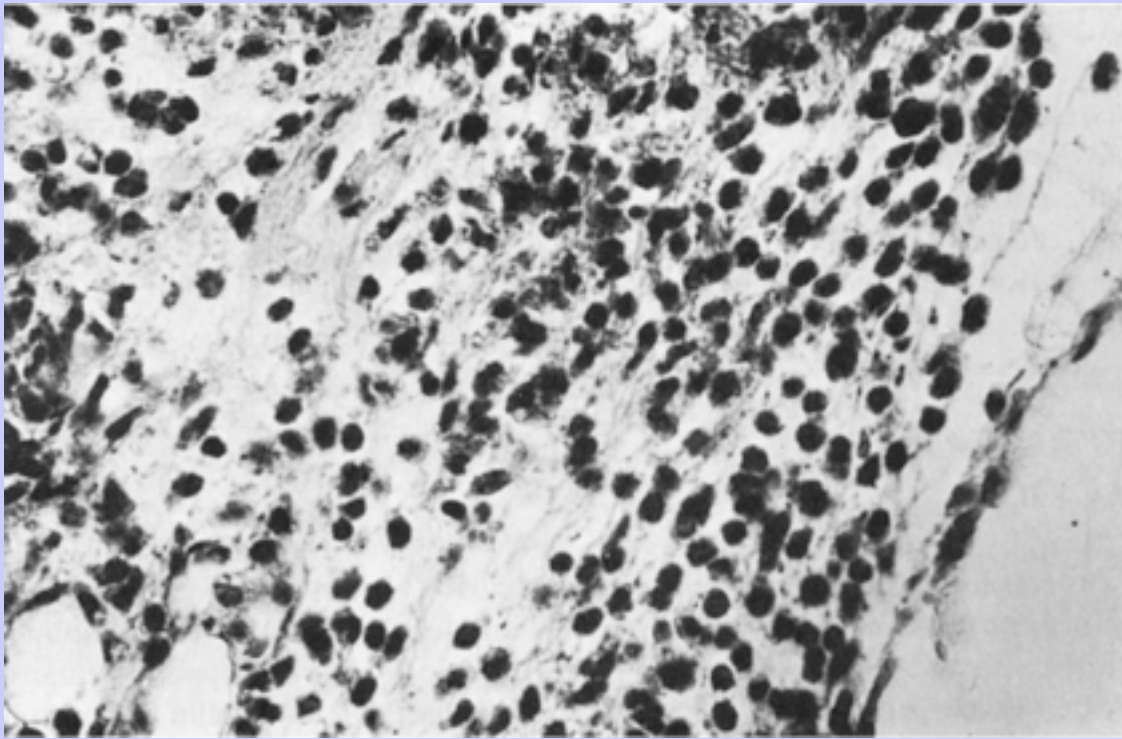


fluctuation in parasite levels, with each peak reflecting the appearance of a new population with new surface glycoproteins, can continue for many months.

Variant surface glycoproteins (VSGs) are the major surface antigens of these trypanosomes. The VSGs produced early in trypanosome infections tend to develop in a predictable sequence. However, as the infection progresses, the production of VSGs becomes more random. Trypanosomes grown in tissue culture also show spontaneous antigenic variation demonstrating that the change in surface VSGs is not induced by antibody. The VSGs form a thick coat on the surface of the trypanosome. When antigenic change occurs, the VSGs in the old coat are shed and replaced by an antigenically different VSG. Analysis of this process indicates that although the trypanosomes possess about 1000 VSG genes, only one VSG gene is active at a time. Antigenic variation occurs as a result of replacing an active VSG gene with one from the silent VSG gene pool. Since only a small part of the tightly packed VSG is exposed to host antibodies, it is not even necessary for the complete molecule to change. Replacement of exposed epitopes is sufficient for effective antigenic variation to occur. Early in infections, complete VSG gene replacement occurs. Later on, partial replacement and point mutations can create new antigenic specificities.

Trypanosomiasis is not the only protozoan infection in which variation of surface antigens is seen. It has also been recorded in infections by *B. bovis*, an intraerythrocyte organism that expresses a variant erythrocyte surface antigen. Other protozoa that show antigenic variation include the plasmodia and the intestinal parasite *Giardia lamblia*.

FIGURE 24-5 The characteristic mononuclear cell infiltration of a delayed hypersensitivity reaction in the skin of a mouse following an intradermal injection of an extract of *Toxoplasma gondii* (toxoplasmin). (Courtesy Dr. C.H. Lai.)



Since parasitic protozoa must evade the immune responses, it is not surprising that they preferentially invade immunosuppressed individuals. Organisms that are normally controlled by the immune response, such as *T. gondii* or *Cryptosporidium bovis*, can grow and produce severe disease in immunosuppressed animals. For this reason, acute toxoplasmosis and cryptosporidiosis commonly occur in humans immunosuppressed for transplantation purposes, for cancer therapy, or with AIDS.

24.2.4

Adverse Consequences

The immune responses against protozoa may cause hypersensitivity reactions that contribute to disease. Type I hypersensitivity is a feature of trichomoniasis and results in local irritation and inflammation in the genital tract. Type II cytotoxic reactions are of significance in babesiosis and trypanosomiasis, in which they contribute to the anemia. In babesiosis, red cells express parasite antigens on their surfaces and are thus recognized as foreign and eliminated by hemolysis and phagocytosis. In trypanosomiasis, either fragments of disrupted organisms or possibly preformed immune complexes bind to red cells and provoke their immune elimination, thus causing anemia. Immune complex formation on circulating red cells is not the only problem of this type in trypanosomiasis. In some cases, excessive immune complex formation can lead to vasculitis and

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glomerulonephritis (type III hypersensitivity; see [Chapter 27](#)). Immune complex lesions are a marked feature of visceral *Leishmaniasis*, as described previously.

Trypanosome infections may cause an enormous increase in the number of IgM-secreting cells, so that very high levels of IgM are found in the blood of infected animals. Some of these antibodies are directed against autoantigens. These include rheumatoid factor-like molecules and antibodies against thymocytes, ssDNA, red cells, and platelets. In *T. congolense*-infected cattle, these polyclonally stimulated B cells are BoCD5⁺. As pointed out earlier (see [Chapter 13](#)), CD5⁺ B1 cells are of a different lineage to conventional B2 cells. The mechanism of this polyclonal B cell activation is unknown.

It is probable that a type IV hypersensitivity reaction contributes to the inflammation that occurs when *Toxoplasma* cysts break down and release fresh tachyzoites. Extracts of *T. gondii* (toxoplasmin), if administered intradermally to infected animals, will cause a delayed hypersensitivity response; this has been used as a diagnostic test ([Figure 24-5](#)).

24.2.5 Vaccination

Successful vaccination against protozoan infections of domestic animals is currently limited to cocci-diosis, babesiosis, *Leishmaniasis*, giardiasis, and theileriosis.

Several live coccidial vaccines may be given to poultry. These vaccines typically contain several species and strains of coccidia. Some consist of virulent, drug-sensitive organisms administered repeatedly in very low doses (trickle infection). Other vaccine strains have been attenuated by repeated passaging through eggs, or they have been selected for precocity. These precocious strains have a decreased prepatent time and, as a result, have a decreased ability to replicate and are thus less virulent. All of these vaccines provide solid immunity to coccidia when applied carefully under good rearing conditions. Nevertheless, the dose of coccidia vaccine must be carefully controlled, and the vaccines must be harvested from the feces of infected birds. Vaccinated birds shed oocysts that are transmitted to other birds in a flock. Because of regional strain variation, vaccination with a specific suspension of live oocysts may not be effective in protecting against field strains in all locations.

A commercial vaccine is available to protect dogs and cats against *Giardia duodenalis*. The vaccine contains disrupted cultured *Giardia* trophozoite extracts administered subcutaneously and protects experimentally challenged dogs and cats against infection and clinical disease.

An effective vaccine is available against *Leishmaniasis*. It contains a key protective antigen, the fucose-mannose ligand. Not only does it prevent the development of the disease but it may also serve as an immunotherapeutic agent, producing clinical improvement in dogs with disseminated disease.

Babesia vaccines consist of tick-borne organisms that parasitize red cells and so cause anemia. Many factors contribute to the resistance of animals against babesiosis, including genetic factors (Zebu cattle are more resistant to disease than European cattle) and age. (Cattle show a significant resistance to babesiosis in the first 6 months of life.) Animals that recover from acute babesiosis are resistant to further clinical disease, and this immunity has been considered to be a form of premunity. It is therefore possible to infect young calves when they are still relatively insusceptible to disease and will thus become resistant to reinfection. The organisms employed for this procedure are first attenuated by repeated passage through splenectomized calves and then administered to recipient animals in whole blood. As might be anticipated, the side effects of this type of controlled infection may be severe, and chemotherapy is commonly required to control them. The transfer of blood from one calf to another may also trigger the production of antibodies against the foreign red cells. These

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antibodies complicate any attempts at blood transfusion in later life and may provoke hemolytic disease of the newborn (see [Chapter 26](#)). In a slightly different approach, cattle can be made resistant to East Coast fever (*T. parva* infection) by infecting them with virulent sporozoites and treating them simultaneously with tetracycline.

Since a primary infection with *T. gondii* will confer strong protective immunity on an animal, protective immunization is a real possibility. Thus in New Zealand, a live *Toxoplasma* vaccine containing the S48 incomplete strain has been used successfully for the control of toxoplasmosis in sheep. The strain was developed by prolonged passage in laboratory mice and has lost the ability to develop bradyzoites or to initiate the sexual stages of the life cycle in cats. It produces protection against a severe challenge for at least 18 months. Unfortunately, the vaccine has a shelf life of only 7 to 10 days and can infect people.

24.3 IMMUNITY TO HELMINTHS

Helminths, like protozoa, have adapted to a parasitic existence and so, out of necessity, must have evolved to overcome or evade the immune responses. Parasitic helminths are therefore not maladapted pathogens but fully adapted obligate parasites whose very survival depends on reaching some form of accommodation with the host. Helminths do not replicate within a host; unlike protozoa, the number of helminths present in an individual will be no more than the number that has gained access to the host. Consequently, they usually cause only mild or subclinical disease. As a rule, they cause morbidity but not mortality. Only when helminths invade a host to which they are not fully adapted or in unusually large numbers does acute lethal disease occur. Indeed, one consistent feature of intestinal nematode infestations is the very wide variation in parasite load within an animal population. Most animals harbor a few worms, but a few animals harbor a lot of worms. The size of the parasite burden in a host is controlled by genetic factors and by the host's response to these parasites. Some animals may be predisposed to a heavy infection as a result of genetic, behavioral, nutritional, or environmental factors.

24.3.1 Innate Immunity

Innate factors that influence helminth infestations include not only host-derived effects but also the influence of other parasites within the same host. The presence of adult worms in the intestine may delay the further development of larval stages of the same species within tissues. For example, calves infected with *Cysticercus bovis* show increased resistance to further infestation by this parasite. Similarly, lambs can acquire resistance to *Echinococcus granulosus* so that multiple dosing with large numbers of ova does not result in the development of massive worm burdens. The original dose of ova may stimulate rejection of subsequent doses. Interspecies competition among helminths for mutual habitats and nutrients in the intestinal tract will also influence the numbers, location, and composition of an animal's helminth population.

Innate factors of host origin that influence helminth burdens include the age, sex, and most important, genetic background of the host. The influence of age and sex on helminth burdens appears to be largely hormonal. In animals whose sexual cycle is seasonal, parasites tend to synchronize their reproductive cycle with that of their hosts. For instance, ewes show a spring rise in fecal nematode ova, which coincides with lambing and the onset of lactation. Similarly, the development of helminth larvae in cattle in early winter tends to be inhibited until spring in a phenomenon called hypobiosis. The larvae of *Toxocara canis* may migrate from an infected bitch to the liver of the fetal puppy, resulting in a congenital infection. Once born, the infected pups can reinfect their mother by the more conventional fecal-oral route.

An example of genetically mediated resistance to helminths is seen in the superior resistance of sheep with hemoglobin A to *Haemonchus contortus* and *Teladorsagia circumcincta* as compared with sheep with hemoglobin B. The reasons for this are unclear, but sheep with HbA mount a more effective self-cure reaction

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and a better immune response to many other antigens as well. Another example is the enhanced resistance to *Cooperia oncophora* seen in Zebu cattle as compared with European cattle. In many cases resistance to parasites is linked to the MHC. Thus cattle possessing BoLA-Aw7 and A36 tend to have low fecal egg counts, whereas animals with Aw3 tend to have high fecal egg counts. Some BoLA haplotypes may also be associated with high antibody levels against *Ostertagia*. The SLA complex has been defined in miniature swine, and its effects on parasite immunity can therefore be assessed. Thus in one study there was a 50% lower muscle larval burden in *Trichinella spiralis*-infected cc minipigs as compared with pigs with the dd or aa haplotype. Minipigs carrying at least one copy of the a allele showed an enhanced ability to kill encysted muscle larvae: 47% of pigs carrying the a allele responded to *Trichinella* as compared to 8% of pigs that lacked this allele. The response was characterized by a predominance of lymphocytes and macrophages in the cellular reaction around each larva.

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Clearly, Th2-mediated responses are the normal responses to parasitic helminths. One feature of Th2 mediated responses is the production of alternatively activated macrophages or M2 cells. Among the molecules produced by these cells is arginase-1. Arginase-1 acts on its substrate L-arginine to produce L-ornithine, which is further metabolized to L-proline, polyamines, and urea. L-arginase production is a common feature in helminth infections. Proline and the polyamines are substrates for collagen synthesis and can induce fibroblast proliferation. The granulomas that develop around pathogenic helminths such as schistosomes appear to be driven by arginase-1 production by M2 cells. Arginase-1 can also reduce the local availability of arginine. This in turn may suppress T cell function and have an antiinflammatory effect.

Chitinases are the enzymes that degrade chitin, which is abundant in helminth cuticles and arthropod exoskeletons. As a result chitinases play a role in resistance to helminth and arthropod parasites. Chitinases are produced by mast cells, macrophages, and neutrophils. Some members of the mammalian chitinase family may lack enzyme activity. Nevertheless they may bind to helminth cuticles and serve as opsonins or chemoattractants.

24.3.2

Acquired Immunity

Helminths present the immune system with a challenge. Unlike bacteria or protozoa, parasitic worms have a thick extracellular cuticle that protects the nematode hypodermal plasma membrane. Some nematodes also have a loose coat that they can readily discard when attacked, ensuring that they cannot be severely damaged by conventional immune defenses. Helminth cuticles cannot be penetrated by the membrane attack complex of complement or by T cell perforins. If the immune system is to successfully combat an invading helminth, it must either use cells that can destroy the intact cuticle or attack them through weak spots on their surface such as their digestive tract. Adult parasitic worms in the intestine are bathed in host enzymes, IgA, and mucin, while their feeding end and alimentary tract encounter effector cells, cytokines, antibodies, and complement. The mechanisms of immunity to helminths are thus different from the mechanisms that control other pathogens.

In general, parasitic worms elicit a very strong Th2 response characterized by production of high levels of IL-4, IgE antibodies, and large numbers of eosinophils and mast cells. The best-analyzed examples of acquired immunity to helminths are those in mice since inbred mouse strains differ widely in their ability to expel intestinal nematodes. This variability is a result of the relative activities of T cell subsets. For example, a consistent predisposition to infection is seen in outbred mice reinfected with *Trichuris trichiura* after their first infection is removed by anthelmintic treatment. Those mice that initially had low worm burdens reacquired low worm burdens, and those that had high worm burdens reacquired high worm burdens. These differences are a result of the helper cell responses mounted by each animal. Mice that expel their parasites mount a predominantly Th2 response. Mice that cannot control their worm burden and become chronically infected mount a Th1 response. The Th2 response is associated with the production of IL-4, IL-10, and IL-13, leading to eosinophil mobilization, intestinal mast cell accumulation, and eventually the production of IgE. Expulsion of

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worms is accompanied by mucosal mast cell infiltration, intestinal eosinophilia, elevated serum IgE, and elevated parasite-specific IgG1 levels. Th2 cytokines also have a direct effect on worm populations. For example, mice that cannot produce IL-4 or IL-13 are much more susceptible to *Trichuris muris* than normal mice. If IL-4 is neutralized by administration of specific antibodies or if the Th1 stimulator IL-12 is administered, mice lose their ability to expel worms and become chronically infected. Likewise, if TNF- α is neutralized, the mice also lose their ability to expel the worms. On the other hand, neutralization of the Th1 cytokines IFN- γ or IL-18 enables chronically infected mice to expel their parasites rapidly. Whether an animal mounts a Th1 or Th2 response depends on the dendritic cells that process the antigen. This in turn appears to depend on the way in which antigen encounters dendritic cells and the set of toll-like receptors activated by the antigen.

Since inbred mouse strains are genetically homogeneous, the variations in resistance to *T. muris* must be due to differences among worms. Is it possible that some worms can trigger DC1 responses whereas others trigger DC2 responses? We know that strains of parasite differ in their ability to trigger Th1 and Th2 responses. This may be due to manipulation of the immune response by each worm. For example, *T. muris* can produce a molecule related to IFN- γ that will suppress Th2 responses and so enhance parasite survival. Alternatively, these differences may be due to parasite dose. Thus low-level infestations of *T. muris* stimulate a Th1 response and the parasites persist. If higher doses of parasites are administered, mice mount a Th2 response and the parasites are expelled. Therefore a threshold of infection is likely critical for the development of resistance.

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γ/δ T cells in the intestinal epithelium may be activated by the presence of intestinal worms without the necessity of conventional antigen processing. In mice, these intraepithelial γ/δ T cells produce IL-4 in response to the nematode *Nippostrongylus brasiliensis*.

Mammals will eventually develop immunity to most helminths after several months. In other words, the ability of the parasite to prevent immunological attack is limited, and eventually the host gains control. One parasite, *Ostertagia*, is an exception. Cattle remain susceptible to reinfection by *Ostertagia* for many months, and immunity that can inhibit the production of viable larvae is not seen until an animal is more than 2 years old. It is not surprising that this is the most economically important bovine parasite.

24.3.3

Humoral Immunity

Because nematodes trigger Th2 responses, IgE levels and eosinophil numbers are usually elevated in parasitized animals. Many helminth infestations are associated with the characteristic signs of type I hypersensitivity, including eosinophilia, edema, asthma, and urticarial dermatitis. For example, pigs infested with *Ascaris suum* show cutaneous allergic reactions to injected parasite antigen, as well as degranulation of intestinal mucosal mast cells. In addition, many helminth infections, such as oesophagostomiasis, ancylostomiasis, strongyloidiasis, taeniasis, and fascioliasis, are accompanied by a positive passive cutaneous anaphylaxis (PCA) reaction to worm antigens (see [Chapter 25](#)).

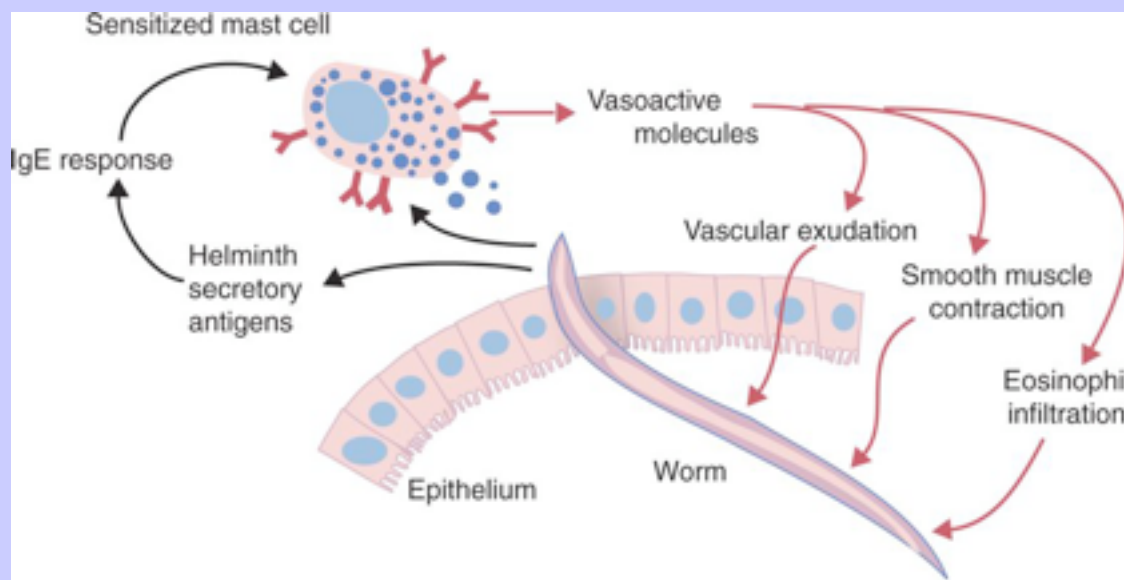
Th2-mediated IgE production is essential in controlling worm burdens. This is well seen in the self-cure reaction in sheep infected with gastrointestinal nematodes, particularly *H. contortus*. While embedded in the intestinal and abomasal mucosa, these worms secrete antigens ([Figure 24-6](#)). The combination of these helminth antigens with mast cell-bound IgE triggers mast cell degranulation and the release of vasoactive molecules, cytokines, and proteases. These molecules stimulate smooth muscle contraction and increase vascular permeability. The Th2 cytokine IL-13 promotes parasite expulsion by stimulating epithelial cell proliferation. Presumably the rapid epithelial cell turnover acts as an “epithelial elevator” to assist in expelling the parasites.

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Violent contractions of the intestinal muscles and an increase in the permeability of intestinal capillaries, leading to an efflux of fluid into the intestinal lumen (a leaky gut), can result in dislodgment and expulsion of many worms. In sheep that have just undergone self-cure, IgE antibody levels are high and experimental administration of helminth antigens will result in acute anaphylaxis, confirming the role of type I hypersensitivity in this phenomenon. A similar reaction is seen in fascioliasis in calves, in which peak PCA antibody titers coincide with expulsion of the parasite.

Macrophages and eosinophils possess FcεR (CD23). These cells can therefore bind to IgE-coated parasites, become activated, and kill them. Thus in cats infected with the filarial worm *Brugia pahangi*, those animals with high levels of parasite-specific IgE can kill adult worms. In contrast, cats that fail to mount a high IgE response permit adult filaria to survive. Macrophages that bind to helminth larvae through IgE become M1

FIGURE 24-6 The mechanisms involved in the self-cure reaction against intestinal helminths. In essence, the animal mounts an allergic response to the salivary antigens of attached nematodes. This acute inflammatory response causes the worms to detach from the intestinal wall and so pass out in the feces.



cells with increased lysosomal enzymes, production of oxidants, IL-1, leukotrienes, prostaglandins, and platelet-activating factor (PAF). The net effect of this is enhanced parasite destruction.

24.3.4 Eosinophils and Parasite Destruction

Eosinophils are attracted to sites of helminth invasion by chemotactic molecules released by degranulating mast cells (Figure 24-7) (see Chapter 25, Table 25-3). Cytokines such as IL-5 from Th2 cells also mobilize the bone marrow eosinophil pool, leading to the release of large numbers of eosinophils into the circulation. Other eosinophil chemoattractants include many chemokines (Figure 24-8). For example, the eotaxins (CCL11, CCL24, and CCL26) have selective chemotactic activity for eosinophils. Parasites may induce two waves of

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eosinophil migration, the first provoked by mast cell or parasite-derived products, the second by IL-5 and other cytokines from Th2 cells. Purified eosinophils exposed to *Strongyloides* antigens significantly increase their expression of CD69 and MHC class II molecules and become antigen-presenting cells. These antigen-presenting eosinophils are highly effective in initiating Th2 responses against worm antigens.

Eosinophils destroy parasitic worms. Because they have Fc receptors, eosinophils can bind to antibody-coated parasites, degranulate, and release their granule contents directly onto the worm cuticle ([Figure 24-9](#)). These contents include oxidants, nitric oxide, and lytic enzymes such as lysophospholipase and phospholipase D. Major basic protein, the crystalline core of the eosinophil-specific granules, can damage the cuticles of schistosomes, *Fasciola*, and *Trichinella*.

FIGURE 24-7 Photomicrograph of a lesion in horse skin caused by allergy to migrating parasitic helminth larvae. The granular cells are eosinophils, and their presence indicates the occurrence of a type I hypersensitivity reaction.

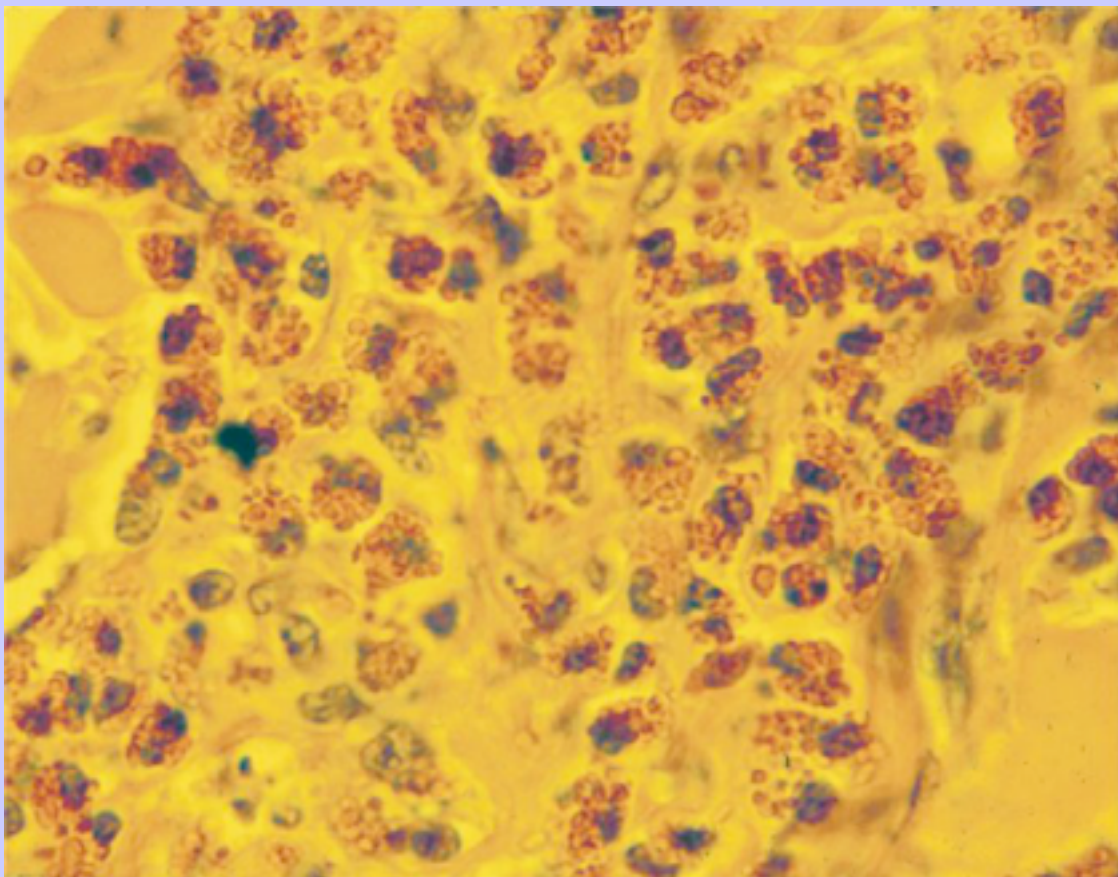


FIGURE 24-8 The factors involved in the activation of eosinophils. As a result of this activation, eosinophils gain enhanced antiparasite functions.

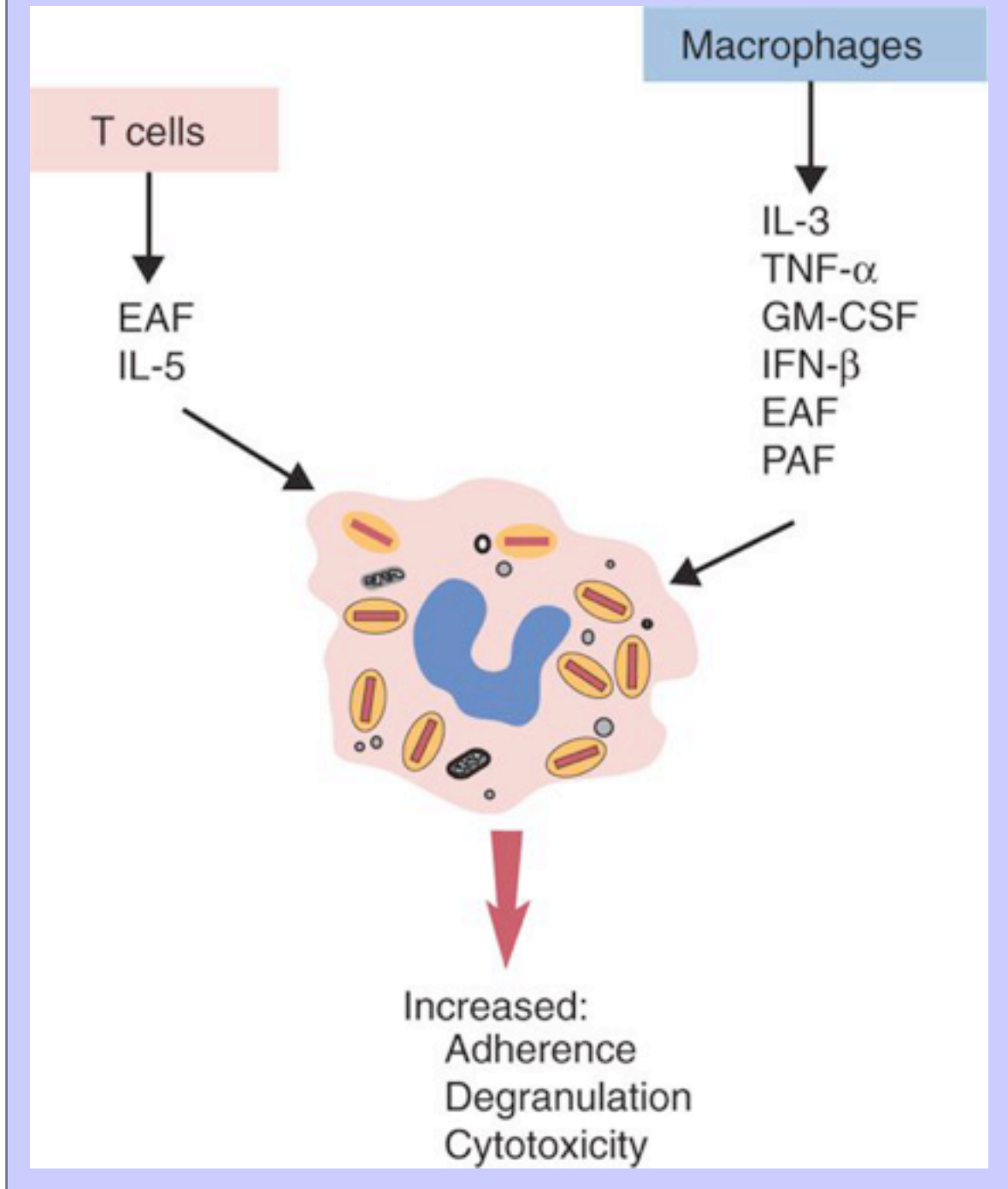
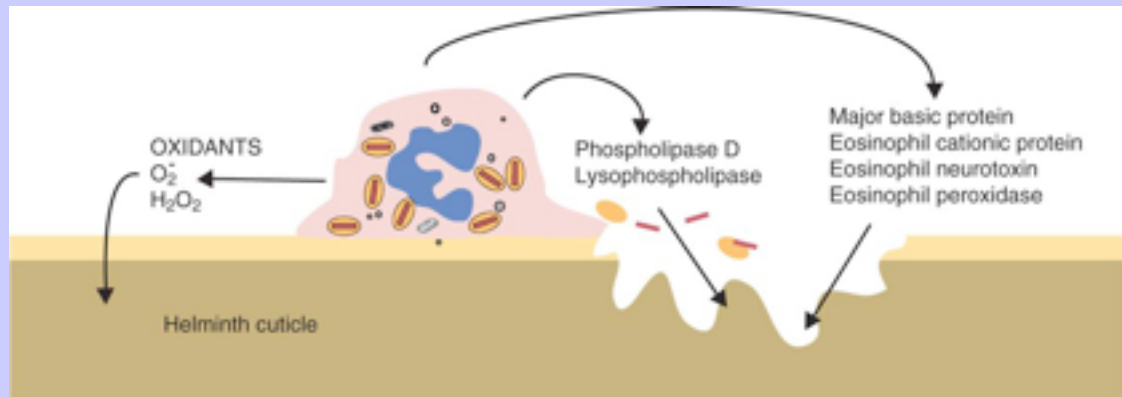


FIGURE 24-9 Some of the molecules released from eosinophils that cause damage to the cuticle of parasitic helminths.



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at very low concentrations. Eosinophil cationic protein and eosinophil neurotoxin are ribonucleases that are lethal for helminths. It is important to point out that eosinophils may not be effective against all parasites. They are probably most effective against larvae in tissues. However, even larval parasites may evade destruction. For example, larvae of *T. canis* exposed to eosinophils simply shed their outer coat together with the attached cells.

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It must also be pointed out that both *Teladorsagia circumcincta* and *H. contortus*, two major parasitic nematodes, produce chemoattractants for eosinophils while the free-living nematode *Caenorhabditis elegans* does not. This challenges the assumption that eosinophils serve a host protective role and, conversely, suggests that some nematodes actively encourage eosinophil recruitment. Perhaps local tissue damage caused by eosinophils provides a suitable microenvironment for parasite invasion.

Although the IgE-dependent eosinophil-mediated response is probably the most significant mechanism of resistance to larval helminths, other immunoglobulins may also play a protective role. The mechanisms involved include antibody-mediated neutralization of larval proteases, blocking of the anal and oral pores of larvae by immune complexes ([Figure 24-10](#)), and prevention of ecdysis and inhibition of larval development by antibodies directed against exsheathing antigens. Antibodies to the enzyme glutathione-S-transferase protect against *Fasciola hepatica* in sheep. Other enzymes may be blocked by antibodies acting against adult worms, stopping egg production, or interfering with worm development ([Figure 24-11](#)). Thus female *Ostertagia ostertagi* worms fail to develop vulvar flaps when grown in immune calves. Similarly, spicule morphology may be altered in *Cooperia* males from immune hosts.

24.3.5

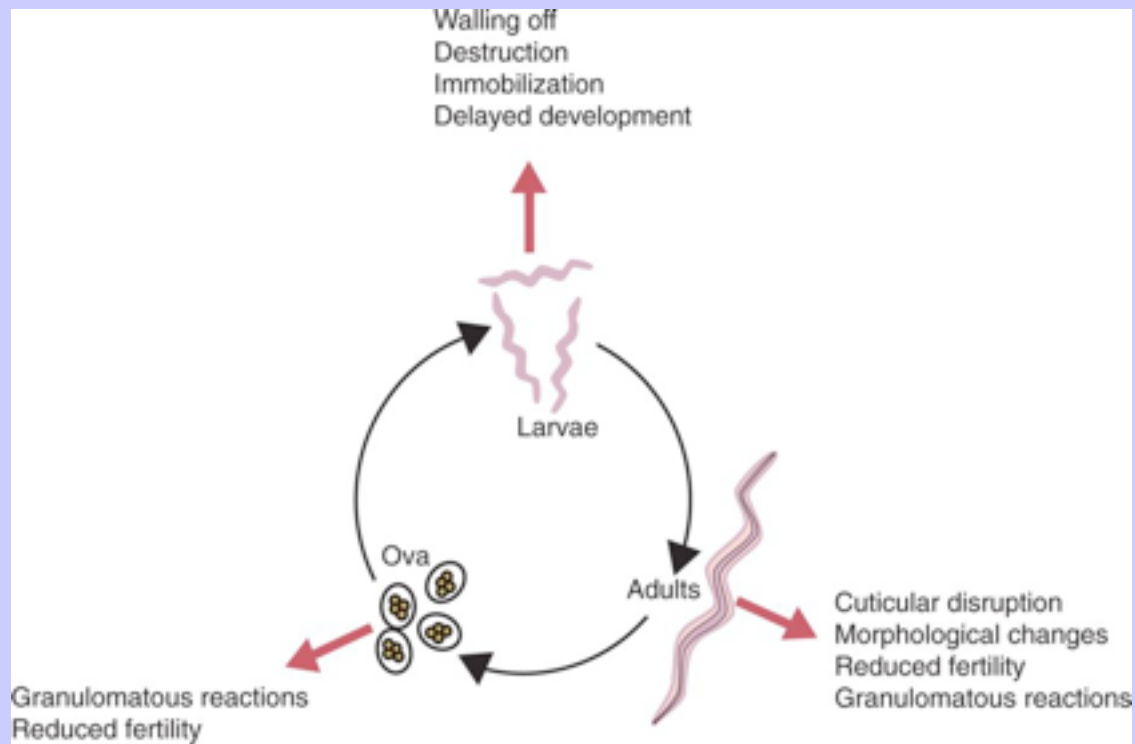
Cell-Mediated Immunity

As pointed out previously, worm antigens preferentially stimulate Th2 responses, and Th1 responses may be of little protective benefit. Nevertheless, cytotoxic T cells may attack helminths that are deeply embedded in the intestinal mucosa or undergoing

FIGURE 24-10 A *Toxocara canis* larva after incubation in specific antiserum. Serum antibodies bind and precipitate antigens in the saliva and excretions of this larva. This precipitate may block these pores and so kill the larva. The immune precipitates at the oral and excretory pores are indicated by arrows. (Courtesy Dr. D.H. DeSavigny.)



FIGURE 24-11 Some effects of the immune responses on the stages of helminth development.



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tissue migration. Cell-mediated immune reactions have been shown to occur in *T. spiralis* and *Trichostrongylus colubriformis* infections. In the former, immunity can be transferred to normal animals by lymphoid cells, and infected animals show delayed hypersensitivity reactions to worm antigens. In vitro tests for cell-mediated immunity such as cytokine production and lymphocyte proliferation are also positive in these infections. In the case of *T. colubriformis*, immunity can be transferred to normal animals from immune ones by both cells and serum, and the site of worm attachment is subjected to a massive lymphocyte infiltration. Lymphocytes from sheep infected with *H. contortus* will release cytokines and divide in response to worm antigen, and it has been shown that immunity to this organism can be adoptively transferred to syngeneic sheep by using immune lymphocytes.

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Live cysts of the tapeworm *Taenia solium* trigger a Th2 response and thus IgE production. However, once the cysts die, they stimulate a Th1 response and granuloma formation. Biopsies show IL-12, IL-2, and IFN- γ associated with granulomas surrounding dying tapeworm cysts. Thus it may be that the Th1 response occurs only when the parasite can no longer modulate the host's immune response.

Sensitized T cells attack helminths by two mechanisms. First, the development of delayed hypersensitivity attracts mononuclear cells to the site of larval invasion and renders the local environment unsuitable for growth or migration. Second, cytotoxic lymphocytes may cause larval destruction. Thus treatment of experimental animals with bacillus Calmette-Guérin vaccine, a treatment that stimulates T cells (see [Chapter 36](#)), inhibits the metastases of hydatid cysts (*E. granulosus*). In these treated animals the space that surrounds the cysts may be

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filled with large lymphocytes. It is also common to observe large lymphocytes adhering firmly to migrating nematode larvae in vivo.

In tapeworm infestations in which the parasite cyst (metacestode) grows within the host, the parasite must obtain protein for nourishment. However, the cysticerci of *Taenia ovis* actually grow larger in the presence of immune serum than in nonimmune serum. The parasites possess Fc receptors for host immunoglobulins, and these immunoglobulins may serve as food for the parasite. Since cyst fluid contains lymphocyte mitogens, it has been suggested that these might stimulate immunoglobulin production that can then be ingested by the parasite.

The complexity of resistance to helminths is well demonstrated in sheep bred for resistance to *H. contortus*. Compared with susceptible sheep, there are differences in B cell function; resistant sheep have significantly more IgA- and IgG1-containing cells. There is also evidence for differences in T cell function because resistant sheep respond better to a T-dependent antigen such as ovalbumin, and treatment of resistant lambs with a monoclonal antibody to CD4 completely blocks their resistance to *H. contortus*. Mucosal mast cell numbers and tissue eosinophilia are also reduced in these treated sheep. In contrast, depletion of CD8⁺ cells has no effect on resistance. In general, resistant sheep have higher eosinophil numbers, and, curiously, these resistant sheep are calmer than susceptible sheep!

24.3.6

Evasion of the Immune Response

Although there are multiple mechanisms whereby animals resist helminth infection, it is obvious, even to a casual observer, that these responses are not very effective. Successfully adapted parasitic helminths can survive and function in the presence of a fully functional host immune system. Several strategies play a role in this adaptation, including loss of anti-genicity by molecular mimicry or absorption of host antigens, antigenic variation, shedding of the glycocalyx, blocking of antibodies, and tolerance.

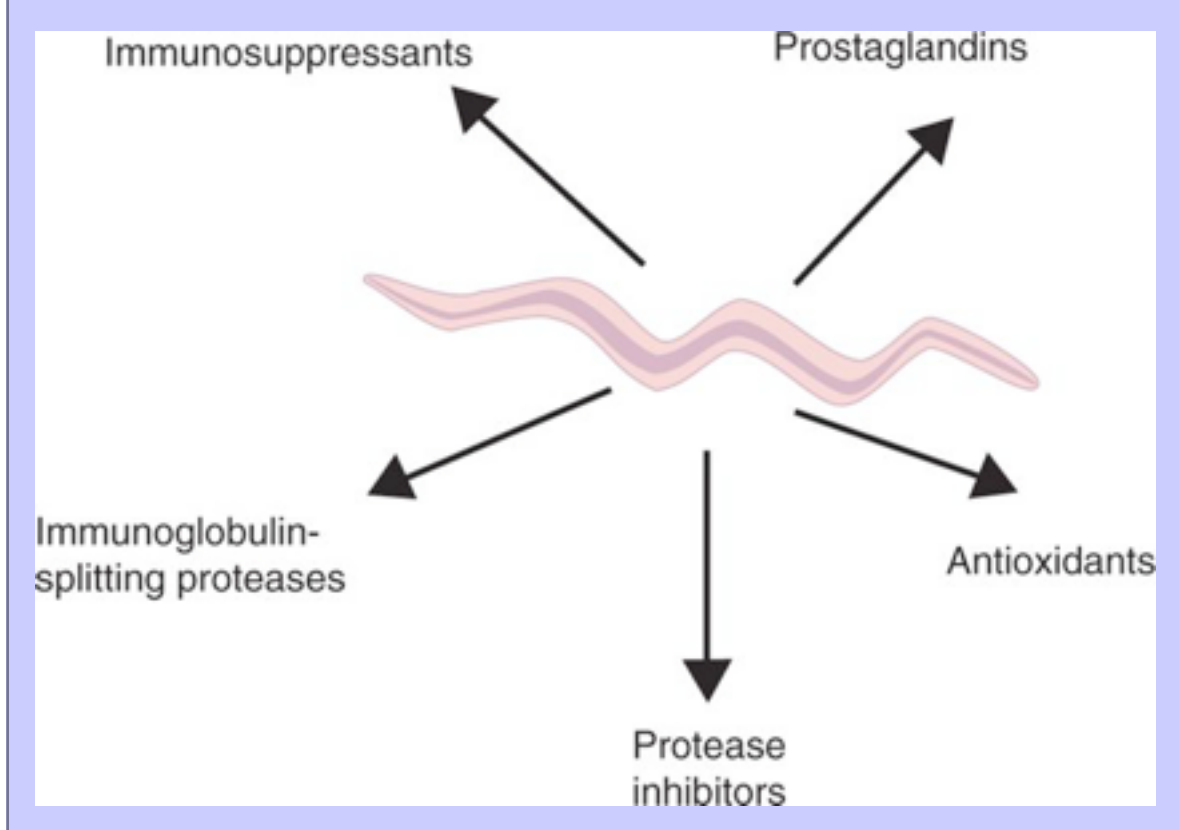
Helminths become progressively less antigenic as they evolve in the presence of a functioning immune system. Presumably, natural selection favors survival of parasites with reduced antigenicity. Thus *H. contortus* has become less antigenic for sheep, its natural host, than for rabbits, which it does not normally infect. Sheep therefore respond to fewer *H. contortus* antigens than do rabbits.

Helminths living within tissues may reduce their antigenicity by adsorbing host antigens onto their surface and so masking parasite antigens. This occurs in *T. solium* infestations in swine, in which the parasites are coated with IgG. It is not clear whether this IgG is synthesized by the worm or whether the worm synthesizes a receptor that binds host IgG. Cysticerci can also adsorb MHC molecules to their surface. Schistosomes can neutralize the alternative complement pathway by inserting functional decay accelerating factor (CD55) from their host into their outer lipid bilayer.

Other helminths interfere with antigen presentation. Thus macrophages from schistosome-infested animals are incompetent antigen-presenting cells. Filarial worms secrete inhibitors that block macrophage proteases. *Taenia taeniaeformis* secretes taeniastatin, a protease inhibitor that inhibits neutrophil chemotaxis, complement activation, T cell proliferation, and IL-2 production. Some parasites such as *F. hepatica* secrete proteases that destroy immunoglobulins. These proteases can generate Fab fragments that bind to parasite antigens and mask them. They may also generate Fc fragments that can block cellular receptors. Tapeworms can interfere with the complement

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FIGURE 24-12 Some methods by which migrating helminth larvae evade host defenses.



system by secreting sulfated proteoglycans, which activate complement in the tissue fluid. *Brugia malayi* secretes serpins that inhibit neutrophil serine proteases. *E. granulosus* secretes an elastase inhibitor that blocks neutrophil attraction by C5a or PAF. Many helminths express surface antioxidants such as superoxide dismutase, glutathione peroxidase, and glutathione-S-transferase, which can neutralize the host's respiratory burst and protect surface structures against oxidation ([Figure 24-12](#)).

Another mechanism of evasion of the immune response involves sequential antigenic variation. Although helminths have not evolved a system similar to that seen in trypanosomiasis, gradual antigenic variation is recognized. Thus the cuticular antigens of *T. spiralis* larvae change following each molt. Even during their growth phase, these larvae change the expression of surface antigens. Some parasites such as *F. hepatica* shed their glycocalyx and hence their surface antigens when exposed to antibodies.

Immunosuppression may also contribute to the survival of parasitic worms. Sheep infected with *H. contortus* may become specifically suppressed so that they are unreactive to *H. contortus*, even though they remain responsive to unrelated antigens. *O. ostertagi* and *Trichostrongylus axei* infestations depress calf lymphocyte responses to mitogens. *Oesophagostomum radiatum* secretes molecules that inhibit the responses of lymphocytes to antigens and mitogens. Other immunosuppressive mechanisms may involve production of suppressor cells, as in filariasis, or, alternatively, immunosuppressive molecules, as in fascioliasis. In other helminth infections, such as trichinosis, infected animals are nonspecifically immunosuppressed. This immunosuppression is reflected in a lowered resistance to other infections, a poor response to vaccines, and a prolongation of skin graft survival.

24.3.7 Vaccination

It is not surprising, considering the poor host response to parasitic worms and the availability of cheap and effective anthelmintics, that antihelminth vaccines are not widely available. Nevertheless, the emergence of anthelmintic resistance and environmental concerns raised by excessive chemical use have resulted in an increased interest in antiparasite vaccines. Vaccine use is predicated on the assumption that a host's immune response can control or prevent an infestation. This is not always obvious in helminth infestations, and traditional vaccines may be of little use. Despite this, a recombinant *T. ovis* vaccine has been produced that can induce protective immunity in sheep. This vaccine contains a cloned oncosphere antigen (To45W) with a saponin-based adjuvant. It stimulates a response that prevents parasite penetration of the intestinal wall. The vaccine provides protective immunity for at least 12 months, and up to 98% of naturally challenged lambs are protected. Similar single-antigen recombinant vaccines have been shown to be highly effective against *E. granulosus* in sheep.

Effective protection against some helminths has been obtained by the use of live irradiated organisms. The most important of these is the vaccine used to protect calves against pneumonia caused by the lungworm *Dictyocaulus viviparus*. In this vaccine, second-stage larvae hatched from ova in culture are exposed to 40,000 R of X-irradiation, and two doses of these larvae are then fed to calves. The larvae can penetrate the calf's intestine, but since they are unable to develop to the third stage, they never reach the lung and are thus nonpathogenic. During their exsheathing process, the larvae stimulate the production of antibodies that can block reinfection. The efficiency of this vaccine, like other vaccines, depends very much on timing and on the size of the challenge dose, since even vaccinated calves may show mild pneumonic signs if placed on grossly infected pastures.

The major helminth antigens are of two types: soluble excretory/secretory products and antigens bound to the parasite surface (somatic antigens). The immunodominant antigens of nematodes are the nematode polypeptide allergens/antigens that act as lipid-binding proteins. Another important somatic antigen is the enzyme α -glutamyl transpeptidase. Some somatic antigens, such as those in the parasite gut, are hidden since they are not normally exposed to the host's immune response and may therefore be potential candidates for vaccines. For example, experimental vaccination of lambs and kids against the intestinal aminopeptidase of *H. contortus* (called H11) has resulted in significant drops in parasite numbers and fecundity. Helminth proteases are also potent inducers of allergic reactions and act directly on mast cells and basophils to induce their degranulation.

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Cattle mount protective responses against *Fasciola* infections. These are especially effective against large infections but are less efficient against low-level trickle infections of the type likely to be encountered in the field. Thus immunity can be transferred from infected to naïve cattle using either lymphocytes or serum. Irradiated parasites may induce immunity, as may crude parasite extracts. Animals may also be protected against fascioliasis by the use of defined parasite antigens, such as fatty acid-binding protein, glutathione-S-transferase, cathepsin L proteases, and liver fluke hemoglobin.

In general, the use of helminth vaccines has not been widely accepted. There appears to be reluctance on the part of farmers to change established control procedures, especially when the major financial burden of these infestations is borne by others.

24.4 IMMUNITY TO ARTHROPODS

When arthropods such as ticks or mosquitos bite an animal, they inject saliva. This saliva contains digestive enzymes that assist the parasite in obtaining its blood meal. The saliva also contains components designed to minimize host responses. For example, saliva may inhibit inflammation. Thus arthropod saliva contains kininases

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that destroy bradykinin, which mediates pain and itch, and histamine-binding proteins that have a similar effect. *Ixodes scapularis* secretes a complement-binding protein that inhibits the generation of C3a. As a result, host scratching and grooming responses are minimized. Because some salivary molecules are antigenic, they induce specific immune responses that may impair the parasite's ability to feed, but the parasites have evolved immunosuppressive countermeasures. Presumably this local immunosuppression and reduced inflammation permit the ticks to feed more effectively. However, they also benefit any parasites injected in tick saliva. Tick salivary immunomodulatory molecules tend, in general, to promote Th2 responses at the expense of Th1 responses. Thus tick saliva impairs macrophage function and suppresses T cell responses to mitogens, as well as production of IL-1 β and the Th1 cytokines IFN- γ and IL-2. It also suppresses NK cell activity and macrophage nitric oxide production. Saliva from the ticks *Dermacentor andersonii* and *Ixodes ricinus* increases production of the Th2 cytokines IL-4 and IL-10. The tick saliva immunosuppressor binds specifically to T cell CD4 and so blocks antigen-induced signaling and T cell responses. Nevertheless, saliva from the tick *I. ricinus* also inhibits host B cell proliferation. A salivary protein from *I. scapularis* inhibits the proliferation of B cells exposed to the Osp proteins from the Lyme disease agent, *B. burgdorferi*, but has no effect on T cells.

Host immune responses to injected arthropod saliva are of three types. Some salivary components are of low molecular weight and cannot function as normal antigens. They may, however, bind to skin proteins such as collagen and then act as haptens, stimulating a Th1 response. On subsequent exposure, these haptens induce a delayed hypersensitivity reaction. Other salivary antigens may bind to epidermal Langerhans cells and induce cutaneous basophil hypersensitivity, a Th1 response associated with the production of IgG antibodies and a basophil infiltration. If the basophils are destroyed by antibasophil serum, resistance to biting arthropods is reduced. The third type of response to arthropod saliva is a Th2 response, leading to IgE production and type I hypersensitivity. This response may induce severe local inflammation in the skin, leading to pain or pruritus. Each of these three types of response may modify the skin in such a way that the feeding of the offending arthropod is impaired and the animal becomes a less attractive source of food. Unfortunately, natural selection and evolution ensure that the biting arthropod is well able to withstand such responses. (These hypersensitivities are discussed further in [Chapter 25](#).)

Immune defenses may play a major role in preventing invasion of skin-penetrating arthropods. Thus body strike results from infestation of sheep skin with the larvae of the fly *Lucilia cuprina*. Sheep can be bred for low and high resistance to body strike. The resistant sheep have greater numbers of IgE⁺ B cells in their skin than do susceptible sheep. Resistant sheep also mount a greater inflammatory response and produce more fluid exudate when injected with larval excretory and secretory products. On the other hand, larval proteases inhibit complement activation and degrade immunoglobulins.

24.4.1 Demodectic Mange

The mange mite *Demodex folliculorum* is a normal symbiont, commonly present in hair follicles, that only occasionally causes disease. When demodectic mange does occur, the reaction around mites and mite fragments is infiltrated by mononuclear cells with a few plasma cells. The infiltrating lymphocytes tend to be CD3⁺ and CD8⁺. Granuloma formation may occasionally occur. Thus the presence of cytotoxic T cells suggests that this is a type IV hypersensitivity reaction, perhaps a form of allergic contact dermatitis. The T cells may also be directed against mite antigens and reflect a defensive response by the host. The absence of eosinophils and edema in the lesion suggests that type I hypersensitivity is relatively unimportant. It is of interest to note that immunosuppressive agents such as antilymphocyte serum, azathioprine, or prolonged steroid therapy predispose animals to the development of demodectic mange. Animals with generalized demodicosis have normal neutrophil function and respond normally to vaccines or other foreign proteins. Nevertheless their T cell

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response to mitogens such as phytohemagglutinin and concanavalin A is depressed. This is a progressive suppression, and it tends to increase in severe cases. If, however, the T cells of dogs with demodicosis are washed free of serum, they regain their ability to respond to mitogens. Serum from these animals is also able to suppress the proliferation of T cells from normal animals.

24.4.2 Flea Bite Dermatitis

Biting fleas secrete saliva into the skin wound. Some of the components of flea saliva are of low molecular weight and act as haptens by binding to dermal collagen. As a result, a local type IV hypersensitivity reaction characterized by a mononuclear cell infiltration occurs. In some sensitized animals, this type IV reaction is gradually replaced over a period of months by a type I reaction, and so the mononuclear cell infiltration gradually changes to an eosinophil infiltration. (A similar series of events has been recorded in sarcoptic mange in swine.) The immune response mounted by flea-allergic animals may have a protective component. Thus female fleas produced fewer eggs on flea-allergic cats than on flea-naïve cats. Flea-allergic cats also appear to remove more fleas by grooming than do flea-naïve animals. Experimental vaccines containing the major antigens from the cat flea midgut have been able to reduce flea populations on dogs, and the female fleas recovered from these immunized animals produced significantly fewer eggs. This suggests that vaccination may eventually be effective in controlling flea populations. Likewise success has been achieved with a recombinant salivary gland protein vaccine to disrupt blood-feeding by horn flies (*Haematobia irritans*). It reduced blood meal size and delayed egg development in flies feeding on vaccinated animals.

24.4.3 Tick Infestation

It has been observed that ticks on nonimmune animals are larger than those on immune animals. Although the nature of this resistance is unclear, it has been suggested that local hypersensitivity reactions to tick saliva may restrict the blood flow to the tick, reduce its food supply, and stunt its growth. It is possible to immunize guinea pigs with tick homogenates and show that ticks feeding on these animals have reduced fertility and egg production. Although vaccination against salivary antigens is unlikely to be very effective in conferring effective immunity against blood-feeding arthropods, there is an alternative approach. Since many of the arthropods of veterinary importance take the blood of their host into their digestive tract, it follows that they will also take up immunoglobulins, complement components, and cells. This suggests that if an animal were immunized with internal antigens from the parasite, this could lead to local damage. These internal antigens have been called “hidden” or “concealed” antigens since under normal circumstances the host would not usually encounter them. Vaccines made against antigens from the intestine of the tick *B. microplus* can inhibit tick reproduction. Indeed, a recombinant tick vaccine based on such a recombinant antigen, Bm86, is available in Australia and Central America. The antibodies produced bind to the brush border of tick intestinal cells, inhibit endocytosis, and prevent the tick from engorging fully. Thus the digestive processes are impaired, and the tick experiences starvation, loss of fecundity, and weakness and may disengage from its host. This lowers the number of ticks found on vaccinated animals.

24.4.4 Hypoderma Infestation

Unlike that of the arthropods described above, the larvae of the warble flies (*Hypoderma bovis* and *Hypoderma lineatum*) actually migrate through body tissues. These larvae are therefore in somewhat the same position as migrating helminth larvae—they must effectively survive or evade the host's xenograft response. In fact, the first instar larvae of these flies do not trigger significant inflammation and are also immunosuppressive. Hypodermin A, the protease secreted by these larvae, can inhibit responses to mitogens and reduce IL-2 production, probably

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by destroying cell surface receptors. Vaccination with a cloned *Hypoderma* protein has effectively protected cattle against subsequent infestations.

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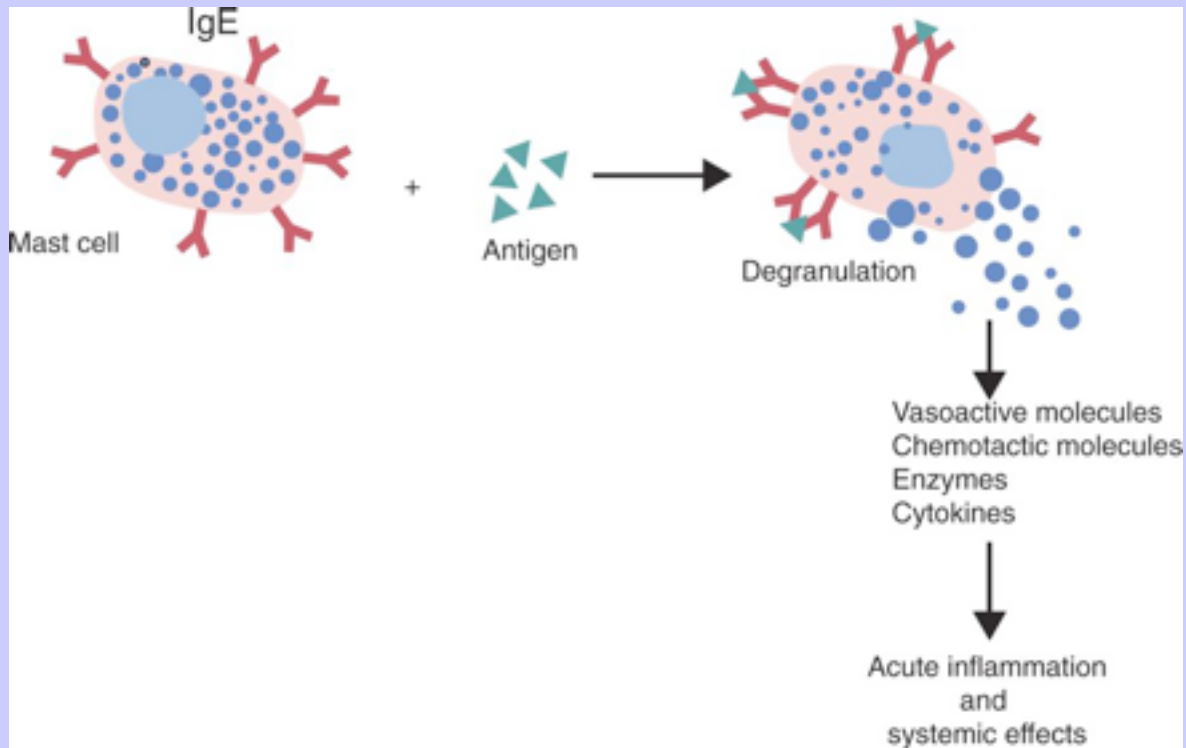
²⁵ CHAPTER 25 Type I Hypersensitivity

^{25.1} KEY POINTS

- Type I hypersensitivities, also called immediate hypersensitivity, are mediated by immunoglobulin E (IgE) attached to mast cells.
- Disease is caused by the rapid release of inflammatory molecules from mast cells following the binding of antigens to IgE.
- The clinical signs of allergic disease depend in large part on the route by which antigens (allergens) enter the body.
- Massive systemic release of inflammatory molecules by mast cells may give rise to allergic anaphylaxis. In this syndrome animals may collapse and die rapidly as a result of the contraction of critical smooth muscles such as those lining the bronchi.
- Animals commonly suffer from allergies to foods, inhaled antigens, vaccines, or drugs.
- In many cases, especially in the dog, these allergies may be manifested by intense pruritus.
- Treatment may include epinephrine for allergic anaphylaxis, corticosteroids for local inflammation, and desensitizing injections of allergen for prolonged control. However, by far the most satisfactory solution is to prevent exposure to the offending allergens.

The role of mast cells in causing acute inflammation was discussed at the beginning of this book. Mast cells serve as sentinel cells. They are covered by an array of receptors that permit them to react in response to many different stimuli. For example, they release inflammatory molecules in response to microbial invasion or tissue damage (see [Chapter 2](#)). This release normally occurs in a controlled manner and ensures that the severity of the inflammation is appropriate to the body's immediate needs. Type I hypersensitivity reactions, in contrast, are a form of inflammation that result from the interaction of antigens with immunoglobulin E (IgE) bound to mast cell IgE receptors. This leads to the rapid release of mast cell secretory granule contents ([Figure 25-1](#)). The granule contents in turn cause acute inflammation.

FIGURE 25-1 The mechanism of type I hypersensitivity reactions. Numerous biologically active molecules are released by mast cells and basophils when antigen cross-links two immunoglobulin E (*IgE*) molecules on the mast cell surface. Some are produced immediately. Others may be synthesized within minutes or hours.



The benefits of this type of inflammation are unclear, but it is of major clinical significance in veterinary medicine ([Box 25-1](#)).

25.2 Box 25-1 Nomenclature

Immunoglobulin E mediates immediate hypersensitivity reactions, so called because they develop within seconds or minutes after exposure to antigen. This type of hypersensitivity reaction is also commonly called an allergy. Antigens that stimulate allergies may be called allergens. If an immediate hypersensitivity reaction is systemic and life-threatening, it is called allergic anaphylaxis or anaphylactic shock. Sometimes an animal may have a reaction that is similar to allergic anaphylaxis but is not immunologically mediated. This type of reaction is described as anaphylactoid.

25.3 INDUCTION OF TYPE I HYPERSENSITIVITY

All animals are exposed to environmental antigens in food and in inhaled air. Most normal animals respond to these antigens by producing IgG or IgA antibodies, and there is no obvious clinical consequence. Some animals,

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however, may respond to environmental antigens by mounting an exaggerated Th2 response and producing excessive amounts of IgE antibodies. These animals develop type I hypersensitivity reactions or allergies. The excessive production of IgE is called atopy, and affected individuals are said to be atopic. The development of atopy and type I hypersensitivity depends on the interaction of genes and environmental factors. The genetics of atopy and allergy are complex. Thus if both parents are atopic, most of their offspring will also be atopic and will suffer from allergies. If only one parent is atopic, the percentage of atopic offspring varies. There is also a breed predisposition to atopy in dogs. For example, atopic dermatitis is most commonly observed in Terriers (Bull, Welsh, Cairn, West Highland White, Scottish), Dalmatians, and Irish Setters, although nonpurebred dogs may also be affected. The heritability of atopic dermatitis in Labrador and golden retrievers is estimated to be a relatively high 0.47. In horses, high levels of IgE are associated with certain major histocompatibility complex (MHC) DRB haplotypes.

Environmental factors such as childhood infections also influence the development of atopic diseases. Thus children who have had multiple infections when young appear to be less likely to develop allergies than those not exposed to such infections. On the other hand, contact with allergens on the first day of life predisposes puppies to develop significantly higher IgE levels than puppies sensitized at 4 months of age.

Normal animals infested by parasitic worms and insects also produce large amounts of IgE. It is believed that the IgE response may have evolved specifically to counteract these organisms. Chitin, the biopolymer that confers structural rigidity to fungi, insects, and helminths, induces the accumulation of cells such as eosinophils and basophils in tissues and may be a key trigger of some of these allergic reactions. Indeed, the self-cure reaction seen in parasitized sheep has long been the only well-characterized beneficial feature of type I hypersensitivity ([Box 25-2](#)) (see [Chapter 24](#)). It is of interest to note that atopic and parasitized dogs may have reduced IgA levels, an observation supporting the concept that a deficiency of IgA may predispose to a compensatory increase in IgE production (see [Chapter 19](#)).

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25.3.1 Box 25-2 Vertical Transmission of Allergies in Dogs?

There is some evidence that the allergic status of parent animals, especially mothers, directly influences the development of allergies in their offspring. Thus in one experiment, two litters of newborn puppies from ragweed-allergic beagles were compared with two litters from nonallergic beagles. (The genetic differences between the parent animals were minimal.) The puppies were repeatedly exposed to ragweed pollen in their inhaled air beginning 1 week after birth. By 40 weeks of age, the puppies from allergic parents had produced high levels of total IgE and ragweed-specific IgE. The puppies from nonallergic parents had produced only IgG antibodies to ragweed. The puppies from allergic parents had eosinophils in their lung washings and developed asthmatic responses to inhaled ragweed pollen. The puppies from nonallergic parents did not. The mechanisms of this effect are unknown. It is possible that factors ingested with the allergic mother's colostrum may favor the switch to a Th2 response in their puppies.

Data from Barrett EG, Rudolph K, Bowen LE, Bice DE: Immunology 110: 493-500, 2003.

25.4 IMMUNOGLOBULIN E

IgE is an immunoglobulin of conventional four-chain structure with a molecular weight of about 200 kDa (see [Chapter 14](#), [Figure 14-7](#)). It is found in serum in exquisitely small quantities (9 to 700 mg/ml in dogs), and its half-life there is only 2 days. Most of the body's IgE is not found in the bloodstream but is firmly bound to Fcε receptors on tissue mast cells, where it has a half-life of 11 to 12 days. Some IgG subclasses may also bind to mast cell receptors and mediate type I hypersensitivity reactions. For example, IgG4 is associated with atopic dermatitis in

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the dog. However, the affinity of these subclasses for mast cells is much lower than that of IgE, and they are of much less clinical significance.

25.4.1 IgE Production

Atopic individuals are predisposed to generate Th2 cells. The Th2 cells produce interleukin-4 (IL-4) or IL-13. These cytokines, together with co-stimulation from CD40, trigger B cell IgE synthesis. IL-4 is also produced in significant amounts by stimulated mast cells. This mast cell-derived IL-4 may alter the helper cell balance and enhance yet more Th2 cell production and IL-4 release ([Figure 25-2](#)). Some allergic humans over-express IL-4, leading to excessive Th2 cell activity and enhanced IgE production.

25.4.2 IgE Receptors

There are two types of IgE receptors: high-affinity FcεRI and low-affinity FcεRII (CD23). There are two forms of FcεRI. One form is found on mast cells, basophils, neutrophils, and eosinophils. This form consists of four chains, one α, one β, and two γ chains (αβγ₂) ([Figure 25-3](#)). The α chain binds IgE, the β chain stabilizes the complex, and the γ chains serve as signal transducers. (This same γ chain is also a signal transducer in FcγRI, FcγRIII, and γ/δ T cell antigen receptor.) The affinity of FcεRI for IgE is very high (10^{-10} M), so they bind almost irreversibly. The presence of FcεRI ensures that mast cells are constantly coated with IgE.

The second form of FcεRI consists of three chains, one α and two γ chains (αγ₂). It is found on antigen-presenting dendritic cells and monocytes. When an antigen binds to this IgE, it is ingested and treated as exogenous antigen. The expression of FcεRI on antigen-presenting cells is enhanced by IL-4 from Th2 cells. Thus a positive feedback loop (the allergy loop) develops ([Figure 25-4](#)). The antigen-processing cells present antigen more effectively to Th2 cells. The Th2 cells then secrete IL-4 and enhance IgE production.

The second type of IgE receptor, FcεRII (CD23), is a selectin found on B cells, natural killer cells, macrophages, dendritic cells, eosinophils, and platelets. In addition to being an IgE receptor, FcεRII also binds the complement receptor CR2 (CD21) ([Figure 25-5](#)). Thus B cells expressing FcεRII will bind CR2 on other B cells, T cells, and dendritic cells. By linking B cells to dendritic cells, FcεRII enhances B cell survival and promotes IgE production.

25.5 THE RESPONSE OF MAST CELLS TO ANTIGEN

When IgE binds to FcεRI on the surface of mast cells, it has no obvious immediate effect on the cell. The mast cell is, however, primed to bind antigen and can reside in tissues with its attached IgE acting like a mine

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FIGURE 25-2 The role of interleukin-4 (*IL-4*) in induction of immunoglobulin E (*IgE*) responses. *IL-4* is produced by Th2 cells. Once released, it promotes the development of more Th2 cells, which are major sources of this cytokine and promote *IgE* responses. The degranulation of mast cells also releases *IL-4*, which further promotes this reaction. Natural killer cells may also serve as an initial source of *IL-4*. The response to *IL-4* is inhibited by interferon- γ (*IFN*- γ) and *IL*-12.

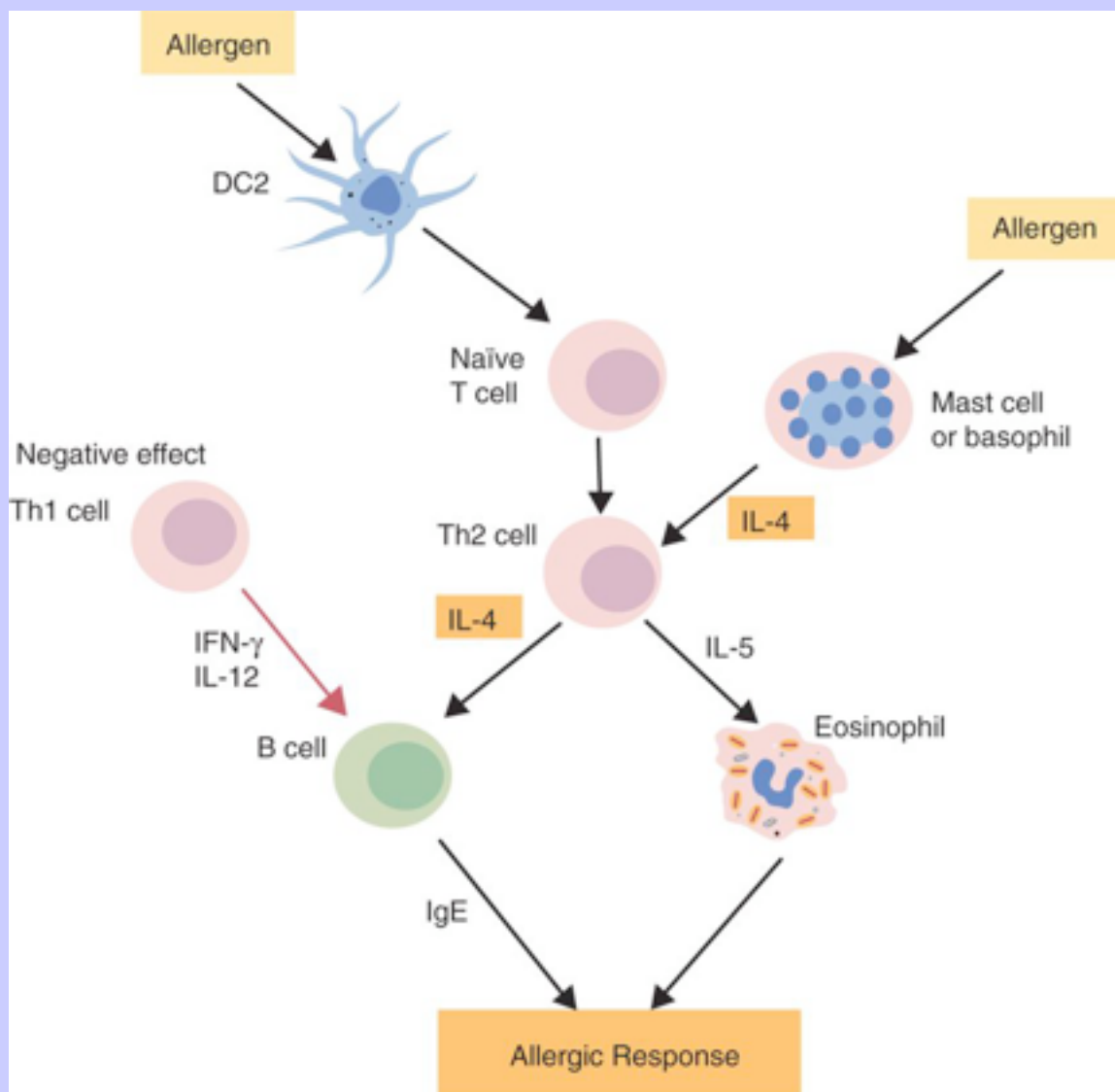
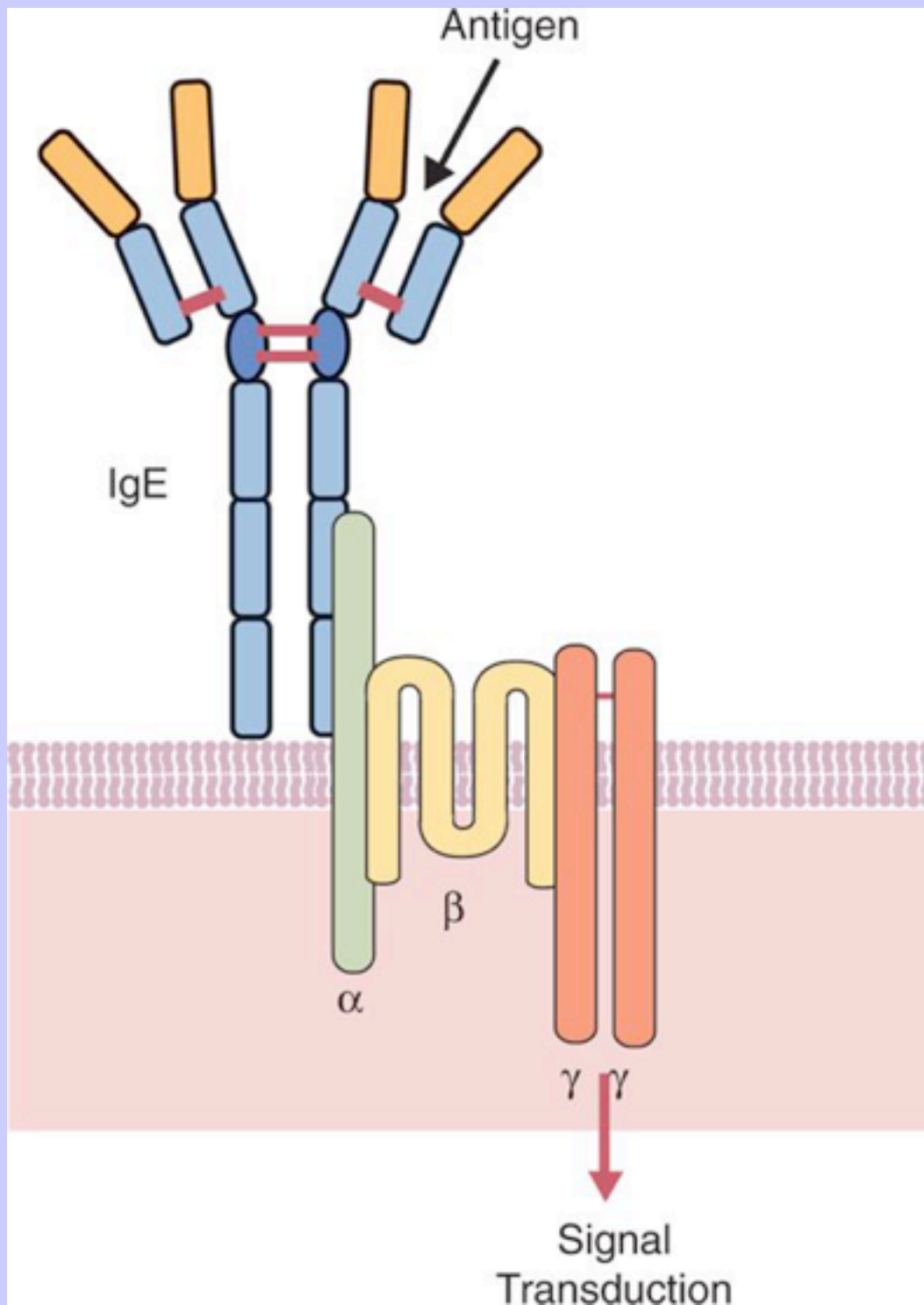


FIGURE 25-3 The structure of FcεRI. The tetrameric form containing two γ chains is found on mast cells and basophils.



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in a minefield. If an antigen enters the tissue, encounters the mast cell, and cross-links two of these bound IgE molecules, the mast cell will be triggered to release the contents of its secretory lysosomes and inflammatory mediators into the surrounding tissues ([Figure 25-6](#)).

This triggering of rapid exocytosis is initiated when an antigen molecule cross-links two FcεRI and activates several protein tyrosine kinases. These, in turn, activate phospholipase C, leading to the production of diacylglycerol and inositol triphosphate. These mediators then increase intracellular calcium and activate more protein kinases. These protein kinases phosphorylate myosin in the cytoskeleton and make the secretory lysosomes move to the cell surface. Their membranes then fuse with the plasma membrane, and their contents are released into the extracellular fluid.

Cross-linking of two FcεRI by an antigen also activates phospholipase A, which acts on membrane phospholipids to produce arachidonic acid. Other enzymes then convert the arachidonic acid to leuko-trienes and prostaglandins (see [Chapter 2](#), [Figure 2-19](#)). Finally, the protein kinases promote transcription

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FIGURE 25-4 The allergy loop. Dendritic cells express trimeric FcεRI and as a result can bind antigen bound to immunoglobulin E (IgE). This antigen, once processed, stimulates Th2 responses. These Th2 cells in turn secrete cytokines, which further promote the IgE response.

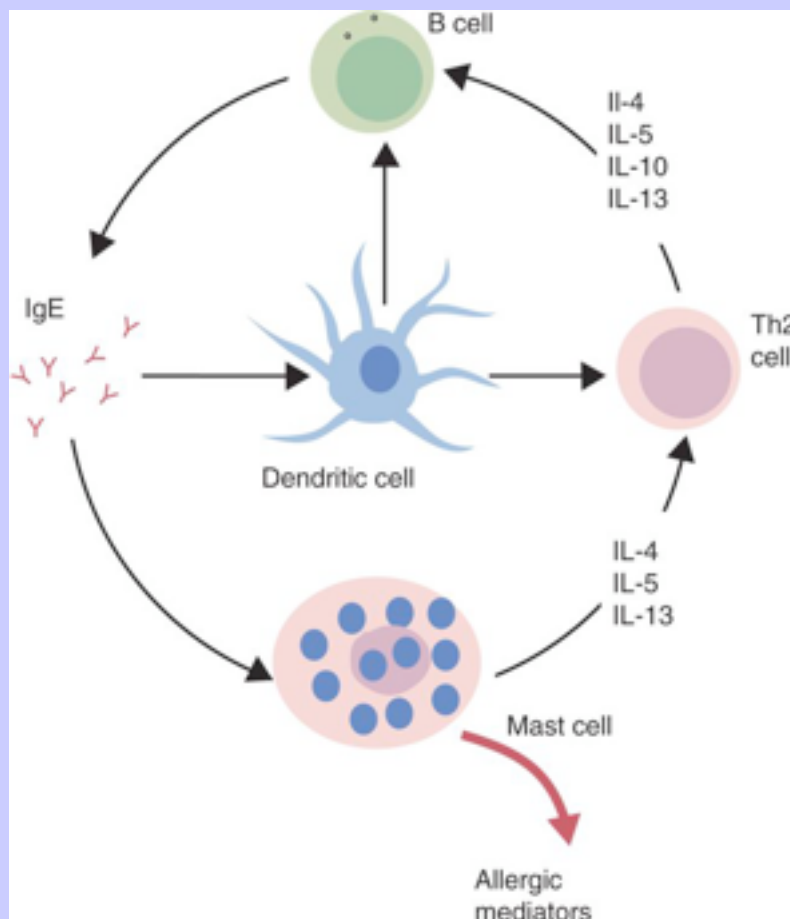


FIGURE 25-5 The combination of the Fcε receptors with their ligands stimulates a variety of different responses in mast cells depending on the nature of these stimuli.

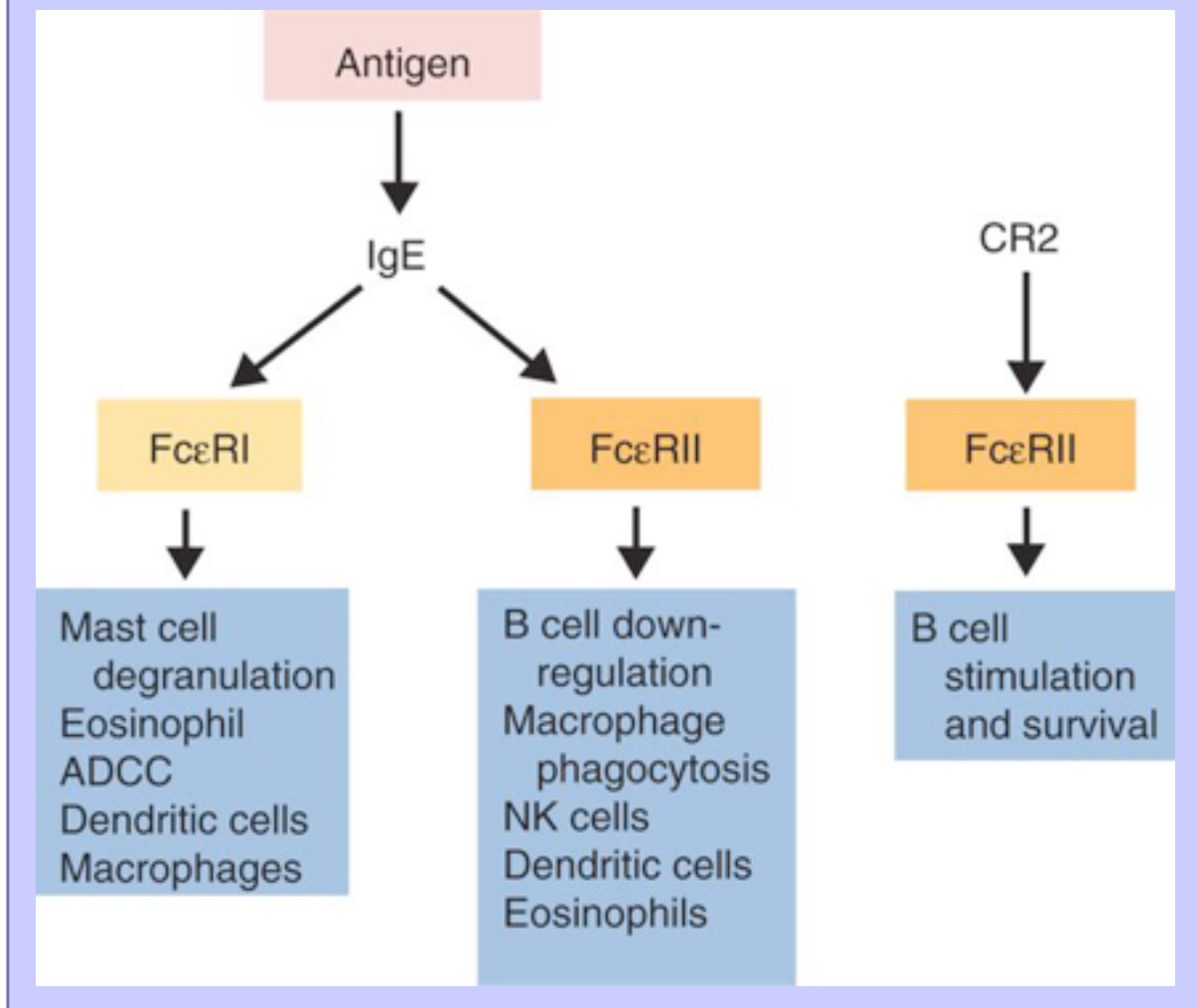


FIGURE 25-6 A simplified view of mast cell signal transduction. The process is triggered by cross-linking two bound immunoglobulin E (*IgE*) molecules with antigen. The combined signal eventually leads to degranulation (granule exocytosis), leukotriene and prostaglandin synthesis, and cytokine production.

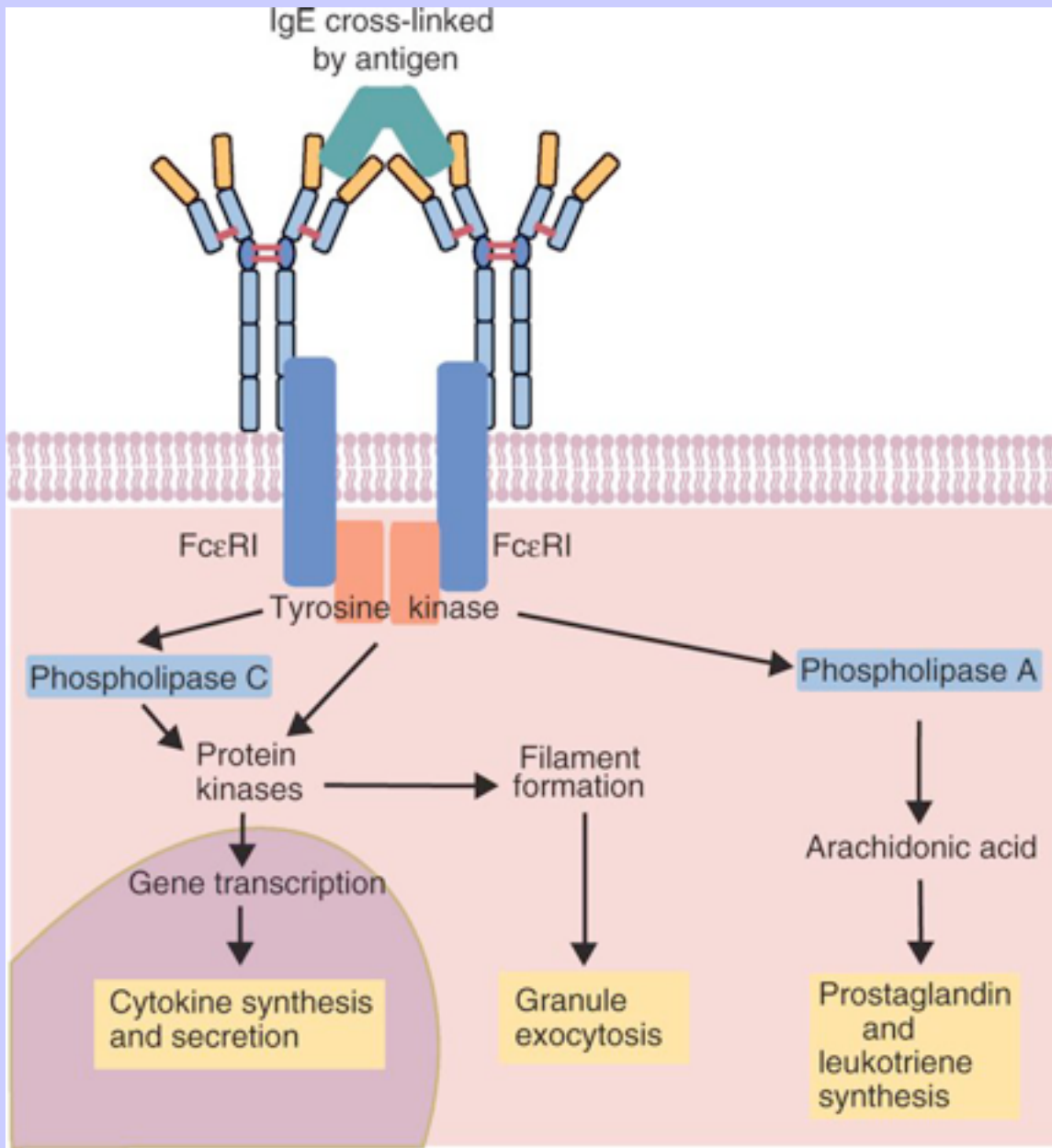
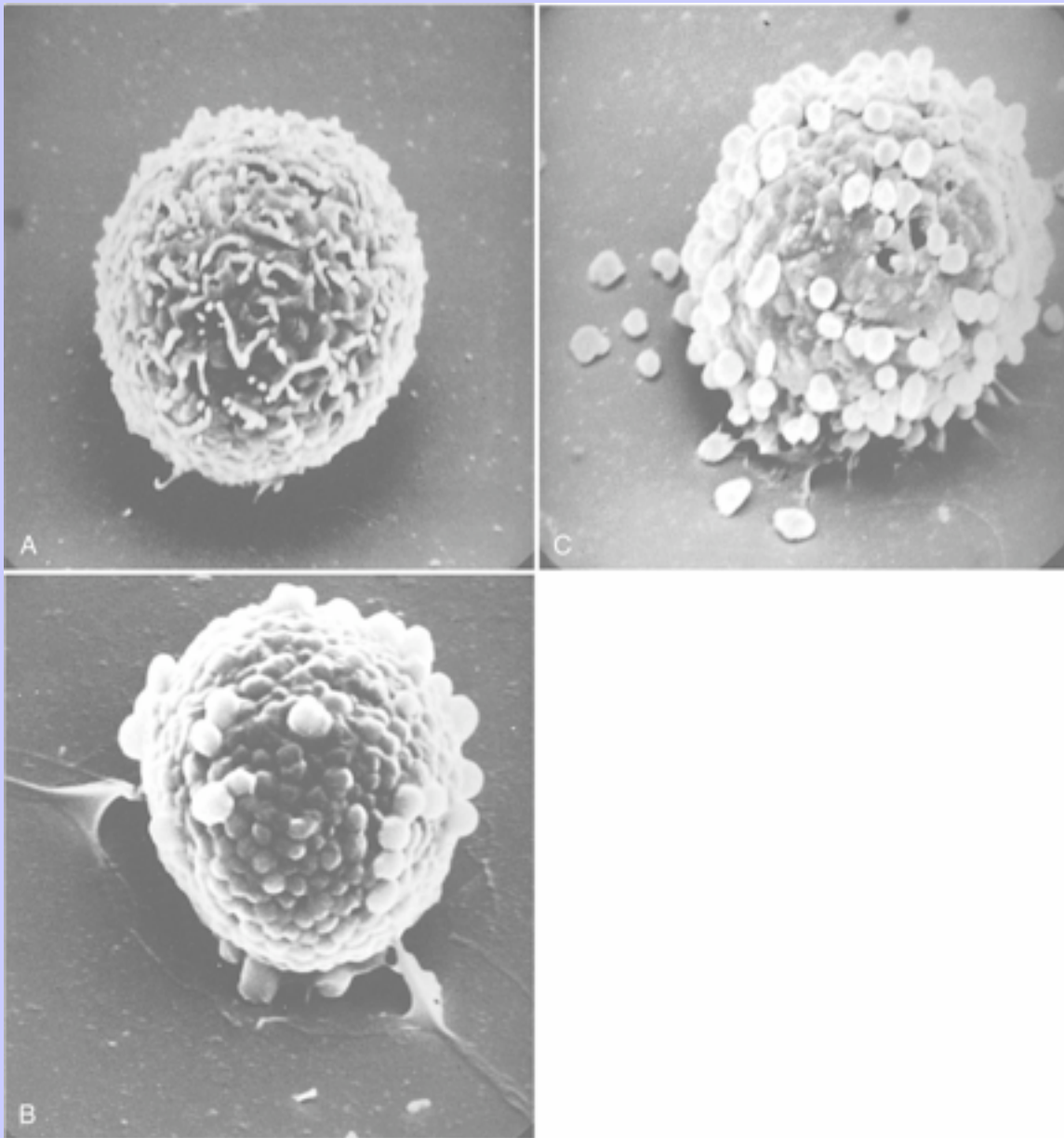


FIGURE 25-7 Scanning electron micrographs showing the rapid exocytosis of granules from stimulated mast cells. **A**, A normal rat mast cell. **B**, A sensitized mast cell fixed 5 seconds after exposure to antigen. **C**, A sensitized mast cell fixed 60 seconds after exposure to antigen ($\times 3000$). (From Tizard IR, Holmes WL: *Int Arch Allergy Appl Immunol* 46:867-879, 1974.)



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and expression of genes coding for many different cytokines as well as the genes for cyclooxygenases and lipoxygenase.

These mast cell responses are extremely rapid. For example, granules are released within seconds after antigen binds to IgE ([Figure 25-7](#)). Because the release is rapid and extensive, sudden acute inflammation develops. Degranulated mast cells do not die but, given time, will regenerate their granules.

25.5.1

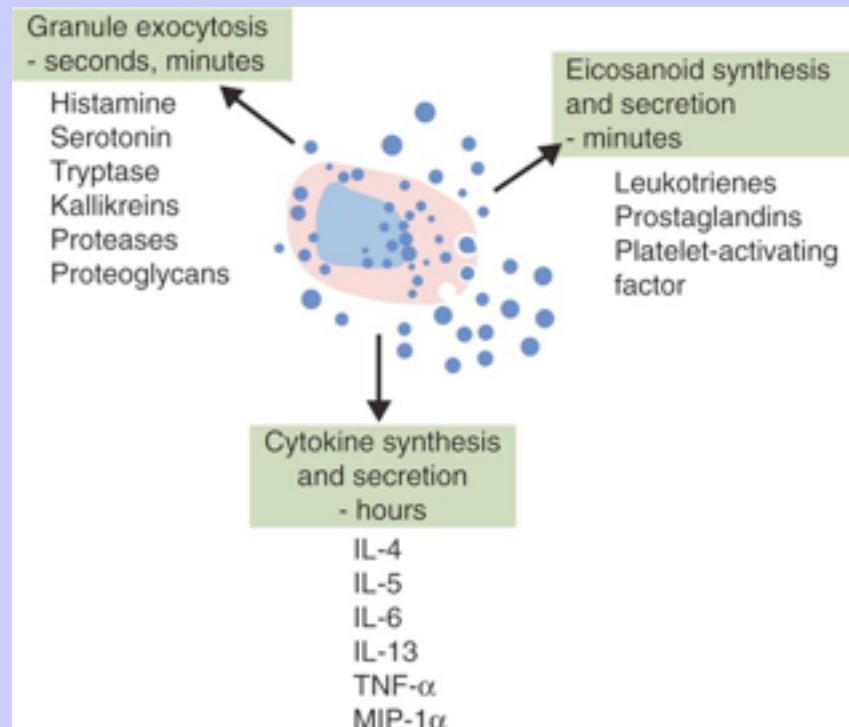
Mast Cell–Derived Mediators

Mast cells are loaded with a complex mixture of inflammatory mediators, enzymes, and cytokines. Triggering of receptor-bound IgE by antigen causes the mast cells to release all these molecules and triggers the productions of many others. All these molecules (both preformed and newly synthesized) generate the acute inflammation characteristic of type I hypersensitivity response ([Figure 25-8](#)). These molecules have been described in detail in [Chapter 2](#). The most important include histamine, serotonin, prostaglandins, and leukotrienes. Mast cells secrete IL-4, IL-5, IL-6, IL-13, IL-16, chitinases, tumor necrosis factor- α (TNF- α), and the chemokine CCL3. These cytokines either are proinflammatory or promote Th2 responses, or both. High levels of these cytokines may therefore be found in tissue fluids in allergic reactions. It is likely no coincidence that mast cells also produce and secrete chitinases. Chitin is characteristically found in insects, fungi, and helminths, and the production of chitinases supports the hypothesis that allergic reactions may have evolved to combat these invaders.

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FIGURE 25-8 The soluble mediators released from degranulating mast cells. These fall into three categories: molecules released from exocytosed granules, lipids (eicosanoids) synthesized within minutes, and proteins synthesized over several hours.



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Table 25-1 Effects of Stimulating α and β Adrenoceptors

System	α Receptor Stimulation or β Blockade	β Receptor Stimulation or α Blockade
Mast cells	Enhances degranulation	Suppresses degranulation
Smooth muscle	Contracts	Relaxes
Blood vessels	Constricts	Dilates

25.5.2 Regulation of Mast Cell Degranulation

Mast cells express two surface receptors for catecholamines called α and β adrenoceptors. These G-protein-linked receptors have opposing effects. Thus molecules that stimulate the α adrenoceptors (such as norepinephrine and phenylephrine) or block the β adrenoceptors (such as propranolol) enhance mast cell degranulation (Table 25-1). In contrast, molecules that stimulate β receptors or block α receptors inhibit mast cell degranulation. β stimulants include isoproterenol, epinephrine, and salbutamol and are widely used in the treatment of allergies. β -receptor blockers enhance mast cell degranulation and promote allergies. Some respiratory pathogens such as *Bordetella pertussis* or *Haemophilus influenzae* can cause β blockade. As a result, the airways of infected animals are more likely to become severely inflamed because of mast cell degranulation. These infections may also predispose animals to the development of respiratory allergies.

25.5.3 Regulation of the Response to Mast Cell Mediators

The α and β adrenoceptors are found not only on mast cells but also on secretory and smooth muscle cells throughout the body. Alpha stimulators cause vasoconstriction and may be of use in treating severe allergic reactions, reducing edema, and raising blood pressure. Beta stimulators mediate smooth muscle relaxation and may therefore reduce the severity of smooth muscle contraction. Pure α and β stimulators are of only limited use in the treatment of allergic diseases because each alone is insufficient to counteract all the effects of mast cell-derived factors. Epinephrine (or adrenalin), on the other hand, has both α and β adrenergic activity. In addition to causing vasoconstriction in skin and viscera, its β effects cause smooth muscle to relax. This combination of effects is well suited to combat the vasodilation and smooth muscle contraction produced in type I hypersensitivity. Ideally, epinephrine should be available whenever potential allergens are administered to animals.

25.5.4 The Late-Phase Reaction

When antigen is injected into the skin of an allergic animal, two distinct inflammatory responses occur. There is an immediate acute inflammatory response that occurs within 10 to 20 minutes as a result of mast cell degranulation. This is followed several hours later by a late-phase reaction, which peaks at 6 to 12 hours and then gradually diminishes. This late-phase reaction is characterized by redness, edema, and pruritus. It is believed that this late reaction results from the release of inflammatory mediators by eosinophils and neutrophils attracted to the site by mast cell-derived chemotactic factors.

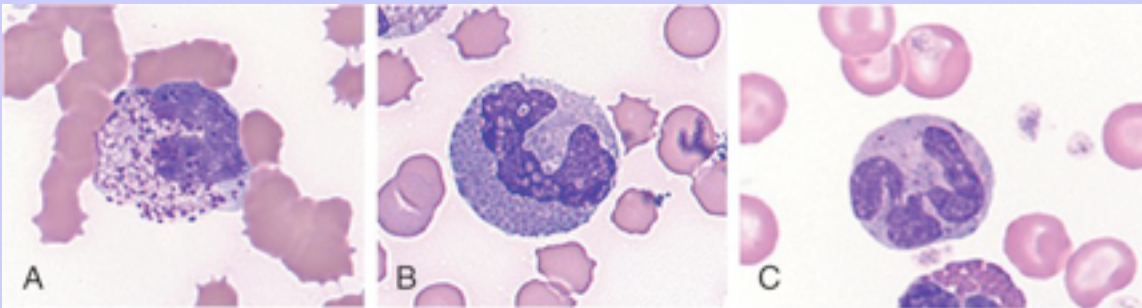
25.6 BASOPHILS

The least numerous granulocytes, the basophils, are so called because their cytoplasmic granules stain intensely with basic dyes, such as hematoxylin ([Figure 25-9](#)). Basophils constitute about 0.5% of blood leukocytes. They are not normally found outside the bloodstream but may enter tissues under the influence of some T cell–derived chemokines. Basophil granules contain a complex mixture of vasoactive molecules similar to those found in mast cells.

The precise relationships between basophils and mast cells have long been a matter of controversy. Thus while the functions of both cell types are clearly

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FIGURE 25-9 Light photomicrographs of peripheral blood basophils from a horse **(A)**, cat **(B)**, and dog **(C)**. These cells are about 10 μm in diameter; all were photographed at the same magnification. Giemsa stain. (Courtesy Dr. M.C. Johnson.)



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very similar, their morphological characteristics and tissue distribution are very different. In mice, basophils and mast cells share a common stem cell. This stem cell is also the source of both connective tissue and mucosal mast cells.

25.7 EOSINOPHILS

Tissues undergoing type I hypersensitivity reactions characteristically contain large numbers of eosinophils. These cells are attracted to sites of mast cell degranulation, where they degranulate and release their own biologically active molecules. Eosinophils may be considered the terminal effector cells of the allergic response.

Eosinophils are polymorphonuclear cells, slightly larger than neutrophils, with cytoplasmic granules that stain intensely with the red dye eosin ([Figure 25-10](#)). They originate in the bone marrow and spend about 30 minutes circulating in the bloodstream before migrating into the tissues, where they have a half-life of about 12 days. The proportion of eosinophils among the blood leukocytes varies greatly since it is affected by the presence of parasites. Normal values range from 2% in dogs to about 10% in cattle.

Eosinophils contain two types of granule ([Figures 25-11](#) and [25-12](#)). Their small, primary granules contain arylsulfatase, peroxidase, and acid phosphatase. Their large crystalloid granules have a core of major basic protein (MBP) surrounded by a matrix containing eosinophil cationic protein, eosinophil peroxidase (EPO), and eosinophil-derived neurotoxin.

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25.7.1 Eosinophil Activation

Three mechanisms are involved in mobilizing eosinophils ([Figure 25-13](#)). First, both Th2 cells and mast cells produce IL-5 and the chemokines known as eotaxins that stimulate the release of eosinophils from the bone marrow. Thus Th2 cells mobilize eosinophils at the same time that they stimulate IgE responses. Second, these eosinophils are attracted to sites of

FIGURE 25-10 Light photomicrographs of peripheral blood eosinophils from a horse (A), cat (B), and dog (C). Each cell is about 12 μm in diameter. Giemsa stain. (Courtesy Dr. M.C. Johnson.)

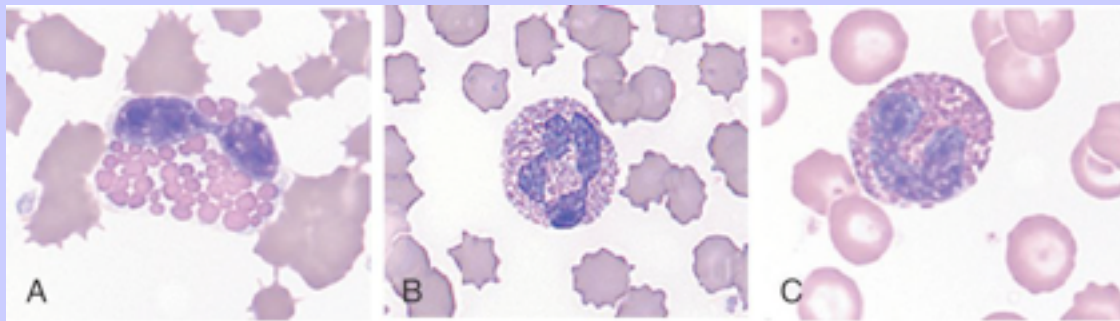
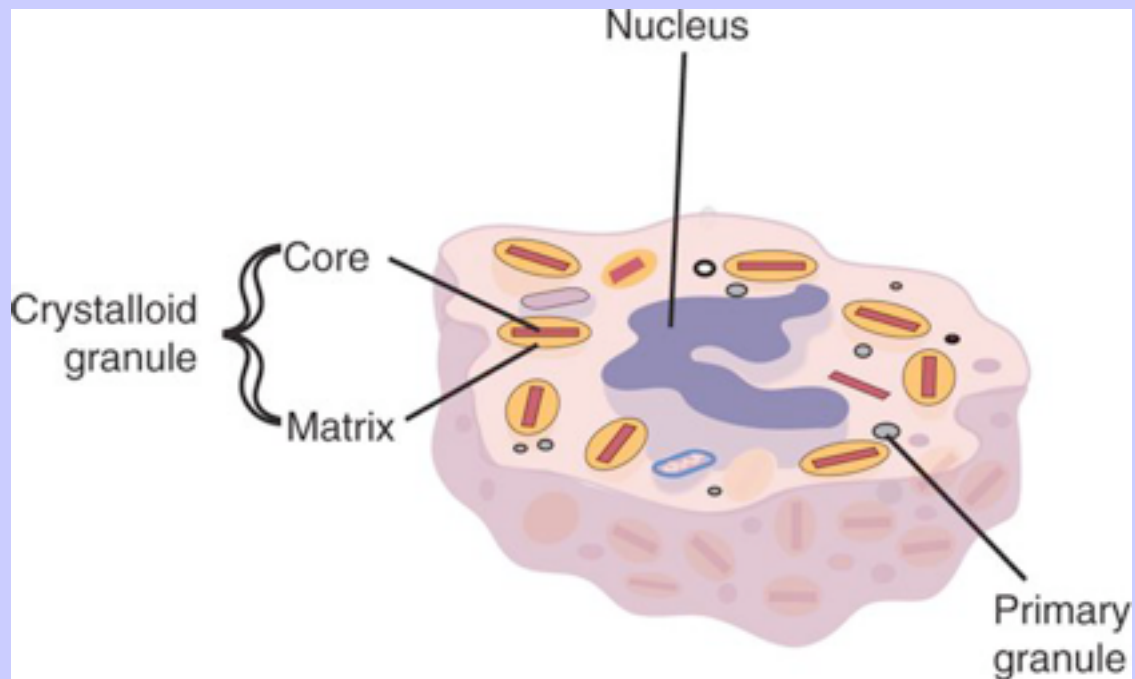
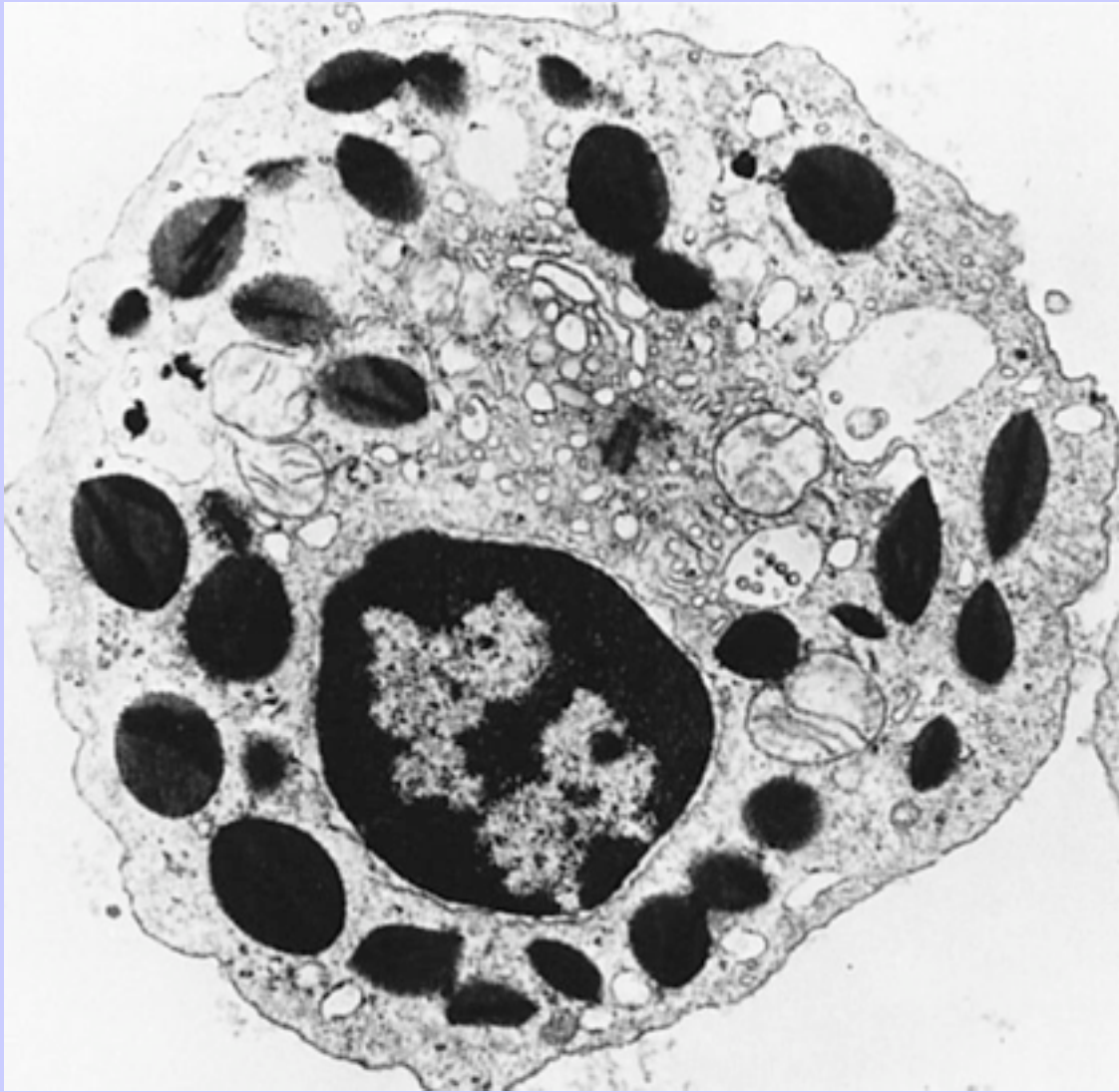


FIGURE 25-11 The major structural features of an eosinophil.



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FIGURE 25-12 A transmission electron micrograph of a rabbit eosinophil.
(Courtesy Dr. S. Linthicum.)

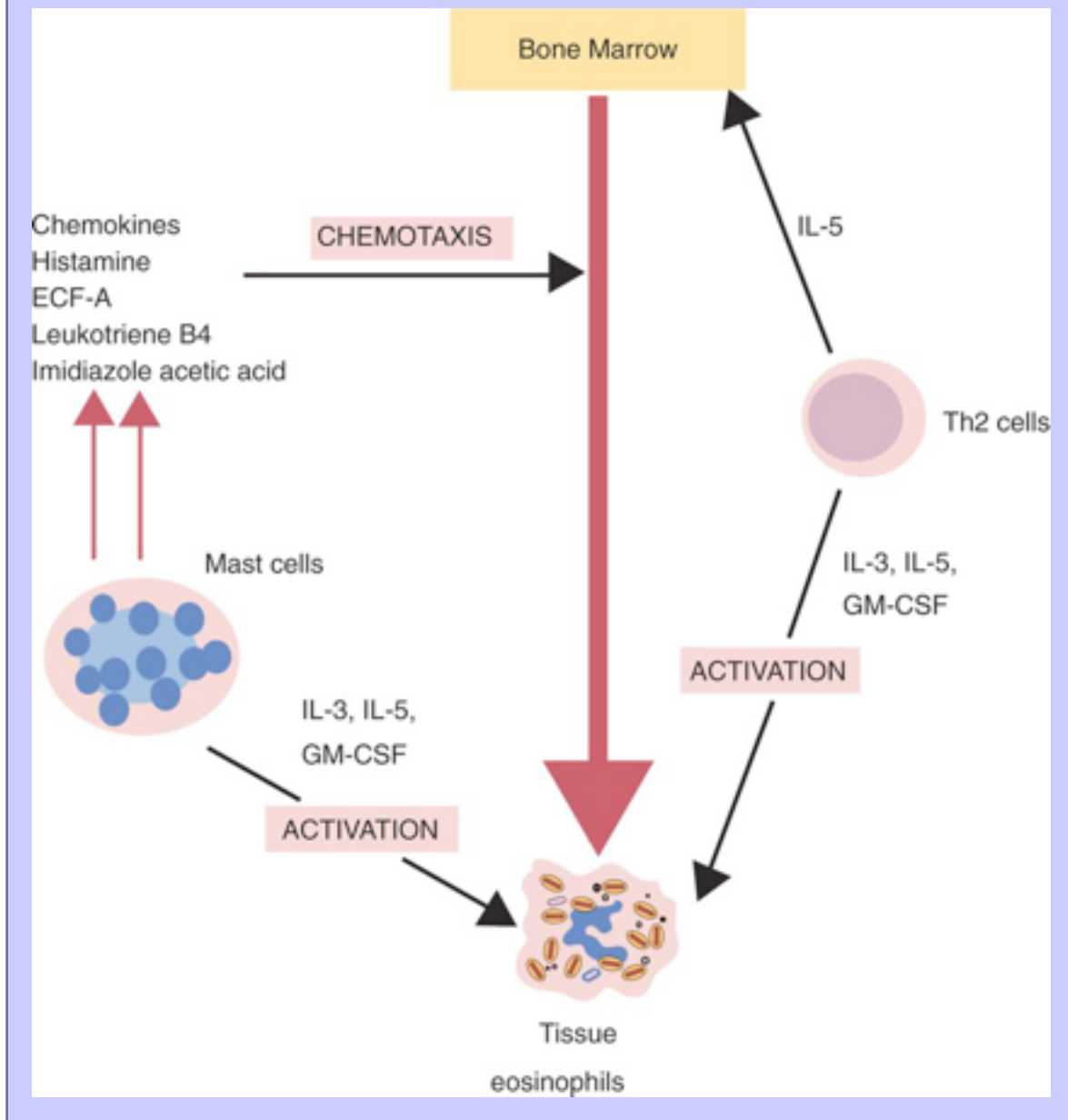


mast cell degranulation by molecules such as the eotaxins, histamine and its breakdown product imidazoleacetic acid, leukotriene B₄, 5-hydroxytryptamine (5-HT), and platelet-activating factor (PAF). Activated eosinophils are especially attracted by CXCL8 (IL-8) complexed to IgA. Third, some common allergens directly activate eosinophils, stimulating their chemotaxis and upregulating CR3 expression. Once they reach sites of mast cell degranulation, the eosinophils are activated by these same molecules. The mobilization and activation of eosinophils enhances their ability to kill parasites and supports the contention that the major function of the IgE-mediated responses is the control of helminth parasites (see [Chapter 24](#)). Activated eosinophils express MHC class II molecules and can serve as antigen-presenting cells. These eosinophils also express the immunosuppressive enzyme IDO (see [Chapter 17](#)). IDO suppresses local Th1 responses but may promote Th2 responses.

25.7.2 Eosinophil Degranulation and Mediators

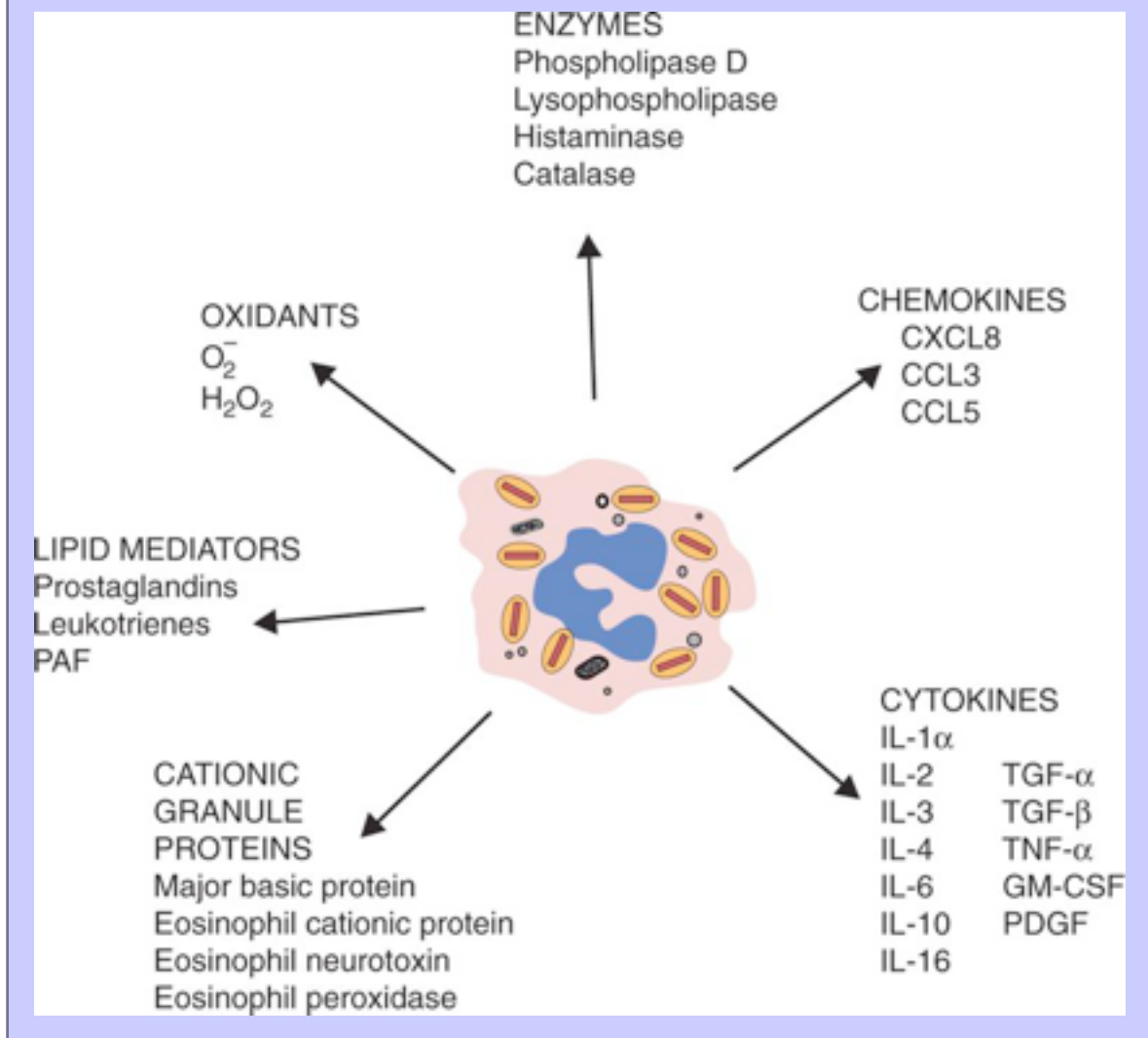
Although eosinophils can phagocytose small particles, they are much more suited to extracellular destruction

FIGURE 25-13 The regulation of eosinophil mobilization, chemotaxis, and activation.



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FIGURE 25-14 Eosinophils release a complex array of molecules that contribute to the acute inflammatory process. It is clear that on balance, eosinophils exacerbate the inflammation triggered by mast cells.



of large parasites since they can degranulate into the surrounding fluid. Eosinophils undergo piecemeal degranulation. In this process small vesicles bud off the secondary granules and are released into the tissues. This degranulation occurs in response to IgE-coated parasites, antigen-bound IgE, many chemokines, PAF, and C5a.

Eosinophil granules contain a mixture of inflammatory and toxic mediators, including cationic proteins, peroxidase, and MBP. Eosinophils also produce lipid mediators such as leukotrienes and PAF. Particles bound to eosinophil receptors trigger a powerful respiratory burst. The EPO uses bromide in preference to chloride, thus producing OBr^- . The peroxidase generates nitric oxide and nitrotyrosine, both potent oxidizing agents.

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The proteins released by degranulation include MBP, cationic protein, and peroxidase. All of these can kill helminths and bacteria and are important mediators of tissue pathology. They all, for example, damage respiratory epithelium. Eosinophils also synthesize and secrete many different cytokines, including IL-1 α , IL-3, IL-4, IL-5, IL-6, granulocyte-macrophage colony-stimulating factor (GM-CSF), TNF- α , transforming growth factor- α (TGF- α), and TGF- β . The production by eosinophils of multiple Th2 cytokines as well as IDO inhibits local Th1 responses and ensures that a “Th2 environment” is maintained in regions of eosinophil accumulation. Eosinophils can produce their own CCL5 and CCL11 so that additional eosinophils can be attracted to the inflammatory focus ([Figure 25-14](#)).

25.7.3 Regulation of Eosinophil Degranulation

Mast cells and eosinophils interact extensively in allergic reactions. Thus eosinophil-derived basic proteins activate mast cells to release histamine. Mast cells in turn release eosinophil chemotactic agents, activate eosinophils, and enhance the expression of eosinophil receptors. Mast cells can synthesize and secrete IL-3, IL-5, and GM-CSF, all of which promote eosinophil degranulation, growth, and survival.

25.8 PLATELETS

While it has long been accepted that immediate hypersensitivity results from the IgE-mediated degranulation of mast cells, it has recently been shown that fatal allergic responses may be induced in mast cell-deficient mice. Analysis indicates that these may be mediated by platelets. Antigen-challenged, sensitized, mast cell-deficient mice failed to show increased vascular permeability in skin but did so in skeletal muscle. The effect was blocked by antiplatelet serum. Given our uncertainties about the pathogenesis of allergic skin disease or anaphylaxis in birds, it seems that studies on the role of platelets in these conditions would likely be rewarding.

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25.9 CLINICAL TYPE I HYPERSENSITIVITY

The clinical signs of type I hypersensitivity result from the abrupt and excessive release of inflammatory mediators from mast cells, eosinophils, and basophils. The severity and location of these responses depend on the number and location of these cells; this, in turn, depends on the degree of sensitization of an animal, the amount of antigen involved, and its route of administration. In its most extreme form, antigen administered rapidly to a sensitized animal will cause generalized mast cell degranulation and massive mediator release. If the rate of release of vasoactive molecules from these mast cells exceeds its ability to respond to the rapid changes in the vascular system, an animal will undergo allergic anaphylaxis and may die.

25.10 ALLERGIC ANAPHYLAXIS

Allergic anaphylaxis is a severe, life-threatening generalized or systemic hypersensitivity reaction. Its precise clinical signs ([Table 25-2](#)) are determined by organ system involvement, which differs among the major domestic animals. Many of the symptoms are a result of vasoactive molecules making smooth muscle contract in the bronchi, gastrointestinal tract, uterus, and bladder.

The major shock organs of horses are the lungs and the intestine. Bronchial and bronchiolar constriction leads to coughing, dyspnea, and eventually apnea. On necropsy, severe pulmonary emphysema and peribronchiolar edema are commonly seen. In addition to the lung lesions, edematous hemorrhagic enterocolitis may cause severe diarrhea. The major mediators of anaphylaxis in horses are probably histamine and serotonin.

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In cattle the major shock organ is the lung. Allergic anaphylaxis is characterized by profound systemic hypotension and pulmonary hypertension. The pulmonary hypertension results from constriction of the pulmonary vein and leads to pulmonary edema and severe dyspnea. The smooth muscle of the bladder and intestine contract, causing urination, defecation, and bloating. The main mediators of anaphylaxis in cattle are serotonin, kinins, and the leukotrienes. Histamine is of much less importance. Dopamine acts in bovine anaphylaxis by enhancing histamine and leukotriene release from the lung, thus exerting a form of positive feedback. Because of the anticoagulant properties of heparin from mast cells, blood from animals

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FIGURE 25-15 Severe urticaria in a Boxer stung by three wasps. (Courtesy Dr. G. Elissalde.)



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experiencing anaphylaxis may fail to coagulate. In cattle, in contrast to the other species, beta stimulants such as isoproterenol potentiate histamine release from leukocytes, whereas alpha stimulants, such as norepinephrine, inhibit histamine release. In addition, epinephrine potentiates histamine release in the bovine. The significance of these anomalous effects is unclear.

Table 25-2 Anaphylaxis in the Domestic Species and Humans

Species	Shock Organs	Symptoms	Pathology	Major Mediators	
Horse	Respiratory tract	Cough	Emphysema	Histamine	
	Intestine	Dyspnea	Intestinal hemorrhage	Serotonin	
		Diarrhea			
Ruminants	Respiratory tract	Cough	Lung edema	Serotonin	
		Dyspnea	Emphysema	Leukotrienes	
		Collapse	Hemorrhage	Kinins	
				Dopamine	
Swine	Respiratory tract	Cyanosis	Systemic hypotension	Histamine	
	Intestine	Pruritus			
Dog	Hepatic veins	Collapse	Hepatic engorgement	Histamine	
		Dyspnea	Visceral hemorrhage	Leukotrienes	
		Diarrhea		Prostaglandins	
		Vomiting			
Cat	Respiratory tract	Dyspnea	Lung edema	Histamine	
	Intestine	Vomiting	Intestinal edema	Leukotrienes	
		Diarrhea			
		Pruritus			
Human	Respiratory tract	Dyspnea	Lung edema	Histamine	
		Urticaria	Emphysema	Leukotrienes	
Chicken	Respiratory tract	Dyspnea	Lung edema	Histamine	
		Convulsions		Serotonin	
				Leukotrienes	

In sheep, pulmonary signs predominate in allergic anaphylaxis as a result of constriction of the bronchi and pulmonary vessels. Smooth muscle contraction also occurs in the bladder and intestine with predictable results. The major mediators of type I hypersensitivity in sheep are histamine, serotonin, leukotrienes, and kinins.

In pigs, allergic anaphylaxis is largely the result of systemic and pulmonary hypertension, leading to dyspnea and death. In some pigs the intestine shows signs of involvement, whereas in others no gross intestinal lesions are observed. The most significant mediator identified in this species is histamine.

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Dogs differ from the other domestic animals in that the major shock organ is not the lung but the liver, specifically the hepatic veins. Dogs undergoing allergic anaphylaxis show initial excitement followed by vomiting, defecation, and urination. As the reaction progresses, the dog collapses with muscular weakness and depressed respiration, becomes comatose, convulses, and dies within an hour. On necropsy, the liver and intestine are massively engorged, perhaps holding up to 60% of the animal's total blood volume. All these signs result from occlusion of the hepatic vein due to a combination of smooth muscle contraction and hepatic swelling. This results in portal hypertension and visceral pooling, as well as a decrease in venous return, cardiac output, and arterial pressure. Identified mediators include histamine, prostaglandins, and leukotrienes.

In cats, the major shock organ is the lung. Cats undergoing allergic anaphylaxis show vigorous scratching around the face and head as histamine is released into the skin. This is followed by dyspnea, salivation, vomiting, incoordination, collapse, and death. Necropsy reveals bronchoconstriction, emphysema, pulmonary hemorrhage, and edema of the glottis. The major pharmacological mediators in the cat are histamine and the leukotrienes.

25.11 SPECIFIC ALLERGIC CONDITIONS

Although allergic anaphylaxis is the most dramatic and severe type I hypersensitivity reaction, it is more common to observe local allergic reactions, the sites of which are referable to the route of administration of antigens. For example, inhaled antigens (allergens) provoke inflammation in the upper respiratory tract, trachea, and bronchi, resulting in fluid exudation from the nasal mucosa (hay fever) and tracheobronchial constriction (asthma). Aerosolized antigen will also contact the eyes and provoke conjunctivitis and intense lacrimation. Ingested antigens may provoke diarrhea and colic as intestinal smooth muscle contracts violently. If sufficiently severe, the resulting diarrhea may be hemorrhagic. Antigen reaching the skin causes local dermatitis. The reaction is erythematous and edematous and is described as an urticarial type (*urtica* is Latin, meaning “stinging nettle”) (Figure 25-15). Urticarial lesions are extremely irritating because of the histamine released; consequently, scratching may mask the true nature of the lesion.

25.11.1 Milk Allergy

Jersey cattle may become allergic to the a casein of their own milk. Normally, this protein is synthesized in the udder, and provided that the animals are milked regularly, nothing untoward occurs. If milking is delayed, however, the increased intramammary pressure forces milk proteins into the bloodstream. In allergic cattle, this may result in reactions ranging from mild discomfort with urticarial skin lesions to acute anaphylaxis and death. Prompt milking can treat the condition, although some seriously affected animals may have to go for several lactations without drying-off because of the severe reactions that occur on cessation of milking.

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25.11.2 Food Allergy

About 2% of ingested protein is absorbed as peptide fragments large enough to be recognized as foreign. This antigen may travel in the blood and reach mast cells in the skin within a few minutes. It has been claimed that up to 30% of skin diseases in dogs are due to allergic dermatitis and that responses to ingested allergens may account for 1% of cutaneous disease in dogs and cats, although its true prevalence is unknown. The clinical consequences of food allergies are seen both in the digestive tract and on the skin.

It is important not to confuse *food allergy*, an immunologically mediated reaction to food allergens, with *food intolerance*. The American Academy of Allergy and Immunology has defined *food intolerance* as “those adverse reactions to foods that are not immunologically mediated.” These reactions can include food idiosyncrasies, in

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which an animal responds abnormally to a food; metabolic reactions, in which a food component affects the metabolism of the animal; pharmacological reactions, in which some food components may act like drugs; and food poisoning, in which the adverse reaction is caused by a toxin or organism.

About 10% to 15% of dogs with food allergies have gastrointestinal problems. The intestinal reaction may be mild, perhaps showing only as an irregularity in the consistency of the feces, or it may be severe, with vomiting, cramps, and violent, sometimes hemorrhagic, diarrhea occurring soon after feeding. About half of affected dogs have a nonseasonal pruritic dermatitis. The skin reactions are usually papular and erythematous and may involve the feet, eyes, ears, and axillae or perianal area. The lesion itself is highly pruritic and is commonly masked by self-inflicted trauma and secondary bacterial or yeast infections. This pruritus tends to respond poorly to corticosteroids. In chronic cases the skin may be hyperpigmented, lichenified, and infected, leading to a pyoderma. A chronic pruritic otitis externa may also develop. The foods involved vary but are usually protein-rich such as dairy products, wheat meal, fish, chicken, beef, or eggs. In pigs, fishmeal and alfalfa have been incriminated. Immunoblots of serum from dogs that are allergic to beef and cow's milk showed that they produce IgE against bovine IgG heavy chains.

Thus, IgG is the major allergen in cow's milk. It is likely that it triggers hypersensitivity to lamb as a result of cross-reactivity with sheep IgG. A second major antigen in lamb and beef extracts has been identified as phosphoglucomutase.

Food allergies have been reported in the horse but are uncommon. Wild oats, white clover, and alfalfa have been recognized as allergens in this species. The most reliable test for suspected food allergies is to remove all potential allergens and then feed a hypo-allergenic diet. These elimination diets usually contain meat and carbohydrates from sources to which the animal is unlikely to have been exposed. Examples include mutton, duck, venison, or rabbit with brown rice or potato. Several commercial hypoallergenic diets are available to facilitate this diagnosis. The diet may be supplemented by adding other ingredients until the allergen is identified by a recurrence of clinical signs. Intradermal skin testing and serological assays (radioallergosorbent test [RAST] or enzyme-linked immunosorbent assay [ELISA]) are of limited usefulness in diagnosing food allergies (p. 344). Treatment involves elimination of the responsible food after correctly identifying it. The development of food allergies may be significantly promoted by the presence of nematode parasites. Thus, an antigen (human serum albumin) was fed to two groups of cats. One group was infected with the roundworm *Toxocara cati*; the other group was worm free. Parasitized cats developed significantly higher levels of antibodies to the antigen. Most importantly, they developed higher levels of IgE antibodies, suggesting that the presence of these parasitic worms in the intestine may well provoke food allergies.

25.11.3 Allergic Inhalant Dermatitis

In dogs and cats, allergy to inhaled environmental antigens most commonly leads to an atopic dermatitis with intense pruritus. Terriers and Dalmatians appear to be predisposed to this, but any breed may be affected. Animals may present with the allergic triad: face rubbing, axillary pruritus, and foot licking, although allergic skin lesions can be found anywhere on the body. The specific lesions are secondary to the pruritus and vary from acute erythema and edema to more chronic secondary changes including crusting, scaling, hyperpigmentation, lichenification, and pyoderma. Some animals may have otitis externa or conjunctivitis. The cutaneous inflammatory infiltrate contains mast cells, γ/δ T cells, dendritic cells, low numbers of eosinophils and neutrophils, and few B cells. The allergens implicated include molds; tree, weed, and grass pollens (especially pollens that are small and light and are produced in very large quantities); house dust mites (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*); animal danders; and fabrics such as wool. Depending on the source of allergen, the atopy may be seasonal. Hypersensitivity to a single allergen is uncommon, and most

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animals develop multiple sensitivities. Diagnosis is based on history and identification of the offending antigens by direct skin testing. Canine allergic dermatitis may be treated by corticosteroids or by hyposensitization therapy. Antihistamines and nonsteroidal antiinflammatory drugs seem to help some of the time, although leukotrienes likely play an important role in this disease.

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Nasolacrimal urticaria (hay fever) is an uncommon manifestation of respiratory allergy in dogs and cats. Pollens usually provoke a rhinitis and conjunctivitis characterized by a profuse watery nasal discharge and excessive lacrimation. If the allergenic particles are sufficiently small, they may reach the bronchi or bronchioles, where the resulting reaction can cause bronchoconstriction, wheezing, and recurrent asthma-like paroxysmal dyspnea. It should be noted that basenji dogs have unusually sensitive airways and experience a disease similar to humans with asthma. Cats are also recognized as suffering from asthma manifested by paroxysmal wheezing, dyspnea, and coughing. Although its pathogenesis has not been clarified, asthmatic cats respond well to corticosteroids and inhaled bronchodilators.

A familial allergic rhinitis characterized by extreme nasal pruritus, violent sneezing, dyspnea, mucoid nasal discharge, and excessive lacrimation has been observed in cattle. Depending on the allergen, it may be seasonal. The antigens involved are inhaled and come from a variety of plant and fungal sources. Diagnosis may be confirmed by skin testing. Nasal granulomas may form in chronically affected cattle. These consist of numerous polypoid nodules, 1 to 4 μm in diameter, situated in the anterior nasal mucosa. The nodules contain large numbers of mast cells, eosinophils, and plasma cells.

25.11.4 Atopic Dermatitis

Atopic dermatitis is a chronic, multifactorial syndrome characterized by chronically inflamed and itchy skin. It is very common in dogs (as many as 15% are affected) and has been recognized in cats, horses, and goats. Canine atopic dermatitis has a major breed predilection, being most common in Retrievers, Setters, Terriers, Beagles, Cocker Spaniels, Boxers, Bulldogs, and Shar-Peis. It is commonly associated with reactions to environmental allergens such as house dust mites, pollens, and molds such as the yeast *Malassezia pachydermatis*. However, the etiology of atopic dermatitis is complex and not all cases are associated with IgE antibodies to environmental antigens. Affected dogs commonly present with pruritus. Initially there may be no obvious skin lesions, but this progresses to diffuse erythema. Chronic licking and scratching leads to hair loss, papules, scaling, and crusting. Hyperpigmentation and lichenification may occur. Skin lesions occur most commonly on the ventral abdomen and in the inguinal and axillary regions. About half of affected dogs have otitis externa. Dogs may develop focal "hot spots." The cellular infiltrate within the lesions contains mast cells, Langerhans cells, and γ/δ T cells. There are low numbers of eosinophils and neutrophils and very few B cells. Secondary bacterial or yeast infections complicate the disease. Depending on the inducing allergen, the disease may be seasonal and relapsing. Once it starts, it tends to get progressively worse unless treated. Control of both allergies and secondary infection is critical.

Atopic dermatitis is provoked by environmental, food, and respiratory allergens that enter animals by the oral, respiratory, or percutaneous routes. The importance of the latter route is reflected by the frequency of lesions on contact areas such as the face, feet, and ears. Affected animals commonly show positive skin test responses to intradermally injected allergens. However, serological assays such as the ELISA or RAST, which measure IgE antibodies to the offending allergens, rarely correlate with disease severity or the levels of IgE in the skin and are of limited usefulness. Blood IgE levels may drop to undetectable levels while levels in skin and skin reactivity remain high. The many false-negative results probably reflect the fact that the immunological reactions such as the presence of reactive Th2 cells and elevated IL-4 levels are largely restricted to affected skin. Affected skin contains more IL-4, IFN- γ , TNF- α , and IL-2 and less TGF- β compared with healthy skin. Allergen avoidance is

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the best treatment. Specific desensitization therapy gives good responses in up to 80% of cases, but secondary problems such as bacterial or yeast (*Malassezia*) infections or flea infestations must also be controlled. It may take several months before the benefits of immunotherapy become apparent. Topical therapy such as bathing with emollient shampoos helps considerably. Antihistamines are of limited usefulness but may be of benefit in mild cases. Sources of essential fatty acids such as n(W)3 (fish oil) and n(W)6 (evening primrose oil) help some cases of atopic dermatitis, possibly by affecting lipid synthesis in the skin and promoting the synthesis of antiinflammatory eicosanoids. Glucocorticoids, such as prednisolone, produce rapid remission but may cause significant side effects. They should be used only as a last resort and given, preferably, by the oral route. Treatment with the prostaglandin analog misoprostol has given encouraging results. The immunosuppressive agents cyclosporine and azathioprine have also been effective in some cases of nonseasonal atopic dermatitis, as has the phosphodiesterase-inhibitor pentoxifylline.

25.11.5 Allergies to Vaccines and Drugs

An IgE response may result from the administration of any antigen, including vaccines. It is most likely to occur in vaccines that contain trace amounts of fetal calf serum, gelatin, or casein. This must always be taken into account when animals are vaccinated. Severe allergies have been associated with the use of killed foot-and-mouth disease, rabies, and contagious bovine pleuropneumonia vaccines in cattle. IgE responses may also occur following administration of drugs. Most drug molecules are too small to be antigenic, but many can bind to host proteins and then act as haptens. Penicillin allergy, for example, may be induced in animals either by therapeutic exposure or by ingestion of penicillin-contaminated milk. The penicillin molecule is degraded in vivo to several compounds; the most important of these contains a penicilloyl group. This penicilloyl group can bind to proteins and provoke an immune response. In sensitized animals, injection of penicillin may cause acute systemic anaphylaxis or milder forms of allergy. Feeding of penicillin-contaminated milk to these animals can lead to severe diarrhea. Allergies to many drugs, especially antibiotics and hormones, have been reported in the domestic animals. Even substances contained in leather preservatives used in harnesses, substances in catgut sutures, or compounds such as methylcellulose or carboxymethylcellulose used as stabilizers in vaccines may provoke allergies.

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25.11.6 Allergies to Parasites

The beneficial role of the IgE–mast cell–eosinophil system in immunity to parasitic worms was first observed in the self-cure phenomenon. Helminths preferentially stimulate IgE responses, and helminth infestations are commonly associated with many of the signs of allergy and anaphylaxis; for example, animals with tapeworms may show respiratory distress or urticaria. Anaphylaxis may be provoked by rupture of a hydatid cyst during surgery or through transfusion of blood from a dog infected with *Dirofilaria immitis* to a sensitized animal.

Allergies are also commonly associated with exposure to arthropod antigens. Insect stings account for many human deaths each year as a result of acute anaphylaxis following sensitization to venom. Anaphylaxis can also occur in cattle infested with the warble fly (*Hypoderma bovis*). The pupae of this fly develop under the skin on the back of cattle after the larvae have migrated through the tissues from the site of egg deposition on the hind leg. Because the pupae are so obvious, it is tempting to remove them manually. Unfortunately, if they rupture during this process, the release of coelomic fluid into the sensitized animal may provoke an anaphylaxis-like response that may kill the animal.

In horses and cattle, hypersensitivity to insect bites may cause an allergic dermatitis variously called Gulf Coast itch, Queensland itch, or sweet itch. The insects involved include midges (*Culicoides* spp.), black flies (*Simulium* spp.), stable flies (*Stomoxys calcitrans*), mosquitoes, and stick-tight fleas (*Echidnophaga gallinacea*). If animals

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are allergic to antigens in the saliva of these insects, biting results in the development of urticaria accompanied by intense pruritus. The itching may provoke severe self-mutilation with subsequent secondary infection that may mask the original allergic nature of the lesion.

In mange due to *Sarcoptes scabiei* in dogs and due to *Octodectes cyanotis* in cats, allergies may contribute to the development of skin lesions. The infested dermis is infiltrated with mast cells, lymphocytes, and plasma cells, and an intradermal injection of mite antigen leads to an immediate wheal-and-flare response. Infested animals may also make precipitating antibodies to mite antigens so that immune complexes may contribute to the development of lesions.

Animals do not inevitably respond to arthropod allergens with a type I hypersensitivity. Thus, responses to Demodex mites and to components of flea saliva may be cell-mediated (type IV hypersensitivity; see [Chapter 28](#)). Flea-bite allergic dermatitis is the single most important allergic skin disease. There is no breed or gender predisposition, but atopic animals as well as those exposed to fleas on an intermittent basis tend to get more severe disease. Continual exposure to fleas at an early age appears to result in a form of hyposensitization. Pruritus is a consistent feature, as is a history of flea infestation. Affected animals, in addition to the characteristic clinical signs, show a reaction to intradermally injected flea antigen. Most positive animals will respond within a few minutes, but up to 30% may show a delayed reaction at 24 to 48 hours. Hyposensitization therapy has not been shown to be successful in treating flea allergy. Flea allergy can be successfully treated only by total flea control.

25.11.7 The Eosinophilic Granuloma Complex

The eosinophilic granuloma complex is a confusing group of clinical conditions associated with various types of skin lesions (ulcer, plaque, granuloma) in cats. Although their cause is unknown, these conditions have been associated with flea or food allergies or inhalant dermatitis. It has been suggested that they are an allergic response to a feline autoantigen. A seasonal form has clearly been associated with mosquito bites. The eosinophilic plaques in the skin are intensely pruritic. As a result, the lesions may be masked by self-inflicted trauma. Histologically they are associated with a local mast cell and eosinophil infiltration, as well as an eosinophilia. Eosinophilic granulomas, in contrast, are not pruritic, and they present as a line of raised pink plaques. Some may present as scattered individual crusted papules. Eosinophilic ulcers are commonly located in the oral cavity or on the lips. Removal of the offending allergen may result in clinical improvement, and corticosteroid treatment is also of benefit. An idiopathic hypereosinophilic syndrome has been described in humans, cats, and dogs. It is characterized by a prolonged, unexplained eosinophilia, the infiltration of many organs with eosinophils, organ dysfunction (affecting especially the heart, but also the lungs, spleen, liver, skin, bone marrow, gastrointestinal tract, and central nervous system), and death. An eosinophilic enteritis may result from canine hookworm infestation.

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25.12 DIAGNOSIS OF TYPE I HYPERSENSITIVITY

The term *hypersensitivity* is used to denote inflammation that occurs in response to normally harmless material. For example, animals normally do not react to antigens injected intradermally. If, however, a hypersensitive animal is given an intradermal injection of an allergen, this provokes local inflammation. Vasoactive molecules are released within minutes to produce redness (erythema) as a result of capillary dilation, as well as circumscribed edema (a wheal) due to increased vascular permeability. The reaction may also generate an erythematous flare due to arteriolar dilation caused by a local axon reflex. This “wheal and flare” response to an allergen reaches maximal intensity within 30 minutes and then fades and disappears within a few hours. A late-phase reaction sometimes

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occurs 6 to 12 hours after injection of an allergen as a result of the release of mediators by eosinophils and neutrophils.

Direct skin testing using very dilute aqueous solutions of various allergens has been widely used for the diagnosis of allergies in animals, especially those with allergic inhalant dermatitis. Following careful intradermal injection of an allergen solution, the site is examined for a local inflammatory response. The results obtained must be interpreted carefully since both false-positive and false-negative responses may occur. For example, the concentration of antigen in commercial skin testing solutions may be too low. Dogs may be up to 10 times less sensitive than humans to intradermal allergens such as pollens, fungi, or danders. False-positive reactions may be due to the presence of preservatives in the allergen solutions. Results of skin testing are affected by steroid treatment. The precise set of allergens used for intradermal skin testing varies among different locations. However, they commonly include an assortment of allergens from trees, grasses, fungi, weeds, danders, feathers, house dust mites, and insects. Intradermal skin testing is less commonly performed in cats because they fail to develop a significant wheal and the reaction is therefore difficult to evaluate.

An experimental technique used to detect IgE antibodies is called the passive cutaneous anaphylaxis (PCA) test. In this test, dilutions of test serum are injected at different sites into the skin of a normal animal. After waiting 24 to 48 hours, the antigen solution is administered intravenously. In a positive reaction, each injection site shows an immediate inflammatory response. The injected antibodies may remain fixed in the skin for a very long period. In the case of the calf, this may be up to 8 weeks. In the PCA test, it is sometimes difficult to detect very mild inflammatory responses. One way to make them more visible is to inject the test animal intravenously with Evans blue dye. The dye binds to serum albumin and does not normally leave the bloodstream. In sites of acute inflammation in which vascular permeability is increased, the dye-labeled albumin enters the tissue fluid and forms a striking blue patch ([Figure 25-16](#)). The size of this patch may be used as a measure of the intensity of the inflammatory reaction.

Serological methods of measuring the level of specific IgE in body fluids include the RAST, western blotting, and the ELISA (see [Chapter 38](#)). These are not subject to clinical bias, but there has been a poor correlation between the results obtained by serology or skin testing and clinical severity. There is also a poor correlation between ELISA results and intradermal testing. Serological assays are especially prone to a high level of false-positive results (low specificity). A negative ELISA will generally rule out atopy. Best results are obtained by testing for individual allergens rather than for groups of allergens. The reasons for this poor correlation between direct IgE measure

FIGURE 25-16 Passive cutaneous anaphylaxis (PCA) reactions in a calf. Several different sera were tested for PCA activity on the flank of a normal calf. (Courtesy Dr. P. Eyre.)



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ments and in vivo methods such as skin testing are debatable but probably reflect the fact that the skin microenvironment is much more complex than that of the bloodstream. Heavily parasitized dogs may have elevated IgE levels, and this may result in false-positive serological results. It is also possible that immunoglobulins of other classes such as IgG4 may contribute to the development of allergic dermatitis in the dog and not be detected by an ELISA employing anti-IgE. For these reasons many veterinary dermatologists prefer skin testing, despite its drawbacks.

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25.13 TREATMENT OF TYPE I HYPERSENSITIVITY

In practical terms, by far the most satisfactory treatment of allergic disease is avoidance of exposure to the allergen. Except for food allergies, however, avoidance may be difficult or impossible. Other treatments such as desensitization therapy may be used (see below). This has the potential to induce stable, long-term remissions, but immunotherapy of this type is not a substitute for avoidance. The principal indications for drug therapy include short-term temporary relief either while waiting to begin immunotherapy or while waiting for it to take effect. Drugs may also be useful for relief of transient recurrences or in animals in which immunotherapy is not possible. Many different drugs are available to treat type I hypersensitivity, although veterinarians tend to employ only a few of these. Corticosteroids are most commonly used to reduce the irritation and inflammation associated with the acute allergic response. These drugs can suppress all aspects of inflammation by inhibiting nuclear factor kappa-B

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activity and so blocking the production of prostaglandins and leukotrienes (see [Chapter 36](#)). Corticosteroids have a considerable palliative effect on chronic type I hypersensitivities, but it is important to remember that these drugs can have serious side effects. They can be immunosuppressive and can increase an animal's susceptibility to infection (see [Chapter 36](#)).

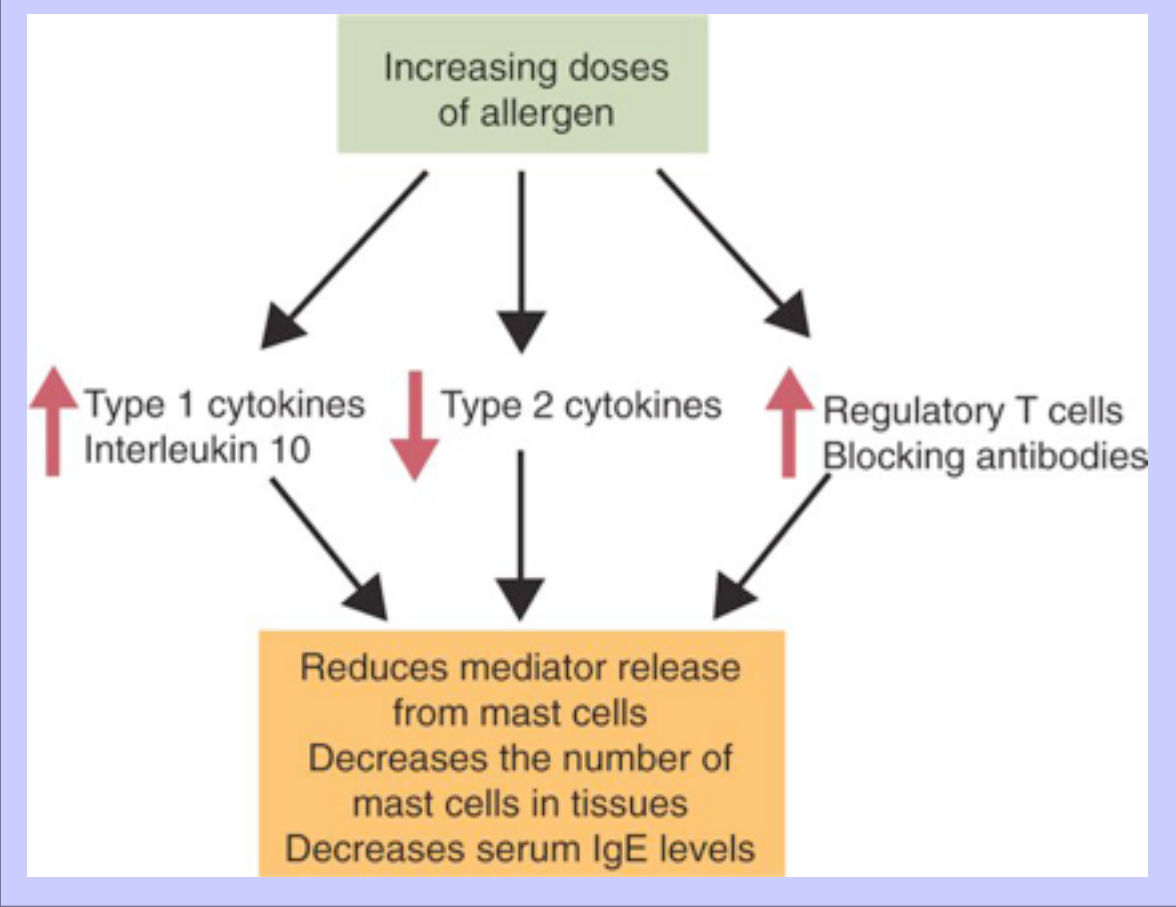
Long-term use of the potent immunosuppressive drug cyclosporine has been reported to be effective and well tolerated as a treatment of canine atopic dermatitis. The skin disease may recur once cyclosporine therapy is terminated.

The β stimulants include epinephrine, isoprenaline, and salbutamol; antagonists include methoxamine and phenylephrine. All have been used extensively in humans and are available for use in animals. Epinephrine is the most important drug used to treat anaphylaxis. It is rapidly absorbed following intramuscular injection and thus can rapidly reverse the clinical signs of shock. Another group of drugs widely employed in the treatment of type I hypersensitivity reactions are the specific pharmacological inhibitors. These drugs, by mimicking the structure of the active mediators, competitively block specific receptors. Thus H1 antihistamines such as diphenhydramine can effectively inhibit the activities of histamine. However, since histamine is but one of a large number of mast cell–derived mediators, antihistamines possess limited effectiveness in controlling hypersensitivity diseases in animals.

25.13.1 Desensitization Therapy

In many animals, allergic disease may be controlled through the use of “allergy shots”—injections of the offending allergen. These injections promote IgG rather than IgE production and reduce the recruitment of inflammatory cells. In humans, immunotherapy of this type reduces mast cell and eosinophil numbers in the lung, as well as the infiltration of CD4⁺ T cells and eosinophils in the skin. Desensitization induces a shift in the dominant helper cell response from Th2 to Th1 cells ([Figure 25-17](#)). For example, the IFN- γ : IL-4 ratio is low in atopic dogs, indicating a Th2 cytokine profile. After specific immunotherapy, the ratio rises significantly as blood IFN- γ levels increase and the balance shifts toward a Th1 response. This IFN- γ blocks the stimulation of IgE antibody synthesis by IL-4 from Th2 cells. The changes in cytokine production promote a shift in allergen-specific immunoglobulin production from IgE to IgG. It is believed that desensitization may activate CD8 T cells that induce dendritic cells to produce IL-12 and IL-18, which synergize in promoting Th1 responses. Desensitization also stimulates T_{reg} cells to produce IL-10, thus inhibiting IgE production,

FIGURE 25-17 The principles of desensitization therapy. Increasing doses of allergen promote a Th1 response, while at the same time reducing the Th2 response and regulating antibody production.



mast cell activation, and histamine and leukotriene release.

In desensitization therapy, small amounts of dilute aqueous solutions of antigen are administered. The first injections contain very little allergen. Over a number of weeks, the dose is gradually increased. If an animal's allergy is seasonal, the course of injections should be timed to reach completion just before the anticipated antigen exposure. It has been estimated that up to 80% of dogs have a good-to-excellent response to desensitization. This would include improvements in clinical signs and a reduction in the amount of medication required. Cats may respond even better. On the other hand, horses with hypersensitivity to biting flies have a poor response to this form of therapy (although, paradoxically, they may show clinical improvement following immune stimulation).

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²⁶ CHAPTER 26 Red Cell Antigens and Type II Hypersensitivity

^{26.1} KEY POINTS

- Type II hypersensitivity, also called cytotoxic hypersensitivity, occurs when an immune response destroys normal cells.
- The destruction of transfused red blood cells when administered to a mismatched recipient is an example of type II hypersensitivity. The disease is a result of the lysis of the transfused red cells by antibodies and complement.
- Mothers may become sensitized by their fetus during pregnancy and so make antibodies against fetal red cells. These antibodies, if ingested in colostrum, may cause destruction of a newborn animal's red cells. This is called hemolytic disease of the newborn.
- Some drugs may bind to blood cells and make them targets of a type II hypersensitivity reaction.

Red cells, like nucleated cells, have cell surface molecules that can act as antigens. However, unlike the major histocompatibility complex (MHC) molecules, red cell surface antigens are not involved in antigen processing, although they do influence graft rejection. (Allografts between blood group-incompatible animals are rapidly rejected.) Most red cell-surface antigens are either glycoproteins or glycolipids and are integral components of the cell membrane that serve key cellular functions. For example, the ABO antigens in humans are anion and glucose transporter proteins, whereas the antigens of the M and C systems of sheep red cells are associated with the membrane potassium pump and amino acid transport, respectively.

If blood is transfused from one animal to another, genetically different individual, the red cell antigens will stimulate an antibody response in the recipient. These antibodies will cause the rapid elimination of the transfused red cells as a result of intravascular hemolysis by complement, and extravascular destruction through opsonization and removal by the mononuclear phagocyte system. Cell destruction by antibodies in this way is classified as a type II hypersensitivity reaction.

^{26.2} BLOOD GROUPS

The antigens expressed on the surface of red blood cells are called blood group antigens or erythrocyte antigens (EAs). There are many different blood group antigens, and they vary in their antigenicity, some being more potent and therefore of greater importance than others. The expression of blood group antigens is controlled by genes and inherited in conventional fashion. Thus for each blood group system there exists a variable number of alleles. (If blood group alleles are invariably inherited together in groups of two or more, they are called phenogroups.) The alleles, in turn, control a variable number of EAs. The complexity of erythrocyte blood group systems varies greatly. They range from simple systems like the L system of cattle, which consists of two alleles controlling a single antigen, to the highly complex B system of cattle. The B system contains several hundred alleles or phenogroups that, together with the other cattle blood groups, may yield millions of unique blood group combinations. Although most blood group antigens are integral cell membrane components, some are soluble molecules found free in serum, saliva, and other body fluids and passively adsorbed onto red cell surfaces. Examples of such soluble antigens include the J antigens of cattle, the R antigens of sheep, the A antigens of pigs, and the dog erythrocyte antigen (DEA) 7 antigens of dogs.

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Animals may make antibodies against foreign blood group antigens, even though they may never have been exposed to foreign red cells. For example, J-negative cattle have anti-J antibodies in their serum, and A-negative pigs have anti-A antibodies. These “natural” antibodies (or isoantibodies) are derived not from previous contact with foreign red cells but from exposure to cross-reacting epitopes that commonly occur in nature (see [Chapter 7, Figure 7-8](#)). Thus many blood group antigens are also common structural components of plants, bacteria, protozoa, and helminths. The presence of these natural antibodies is not, however, a uniform phenomenon, and not all blood group antigens are accompanied by the production of natural antibodies to their alternative alleles.

26.3 BLOOD TRANSFUSION AND INCOMPATIBLE TRANSFUSIONS

Blood is easily transfused from one animal to another. If the donor red cells are identical to those of the recipient, no immune response results. If, however, the recipient possesses preexisting antibodies to donor red cell antigens, they will be attacked immediately. These preexisting antibodies are usually of the immunoglobulin M (IgM) class. When these antibodies bind red cell antigens, they may cause agglutination or hemolysis, or stimulate opsonization and phagocytosis of the transfused cells. In the absence of preexisting antibodies, foreign red cells will stimulate an immune response in the recipient. The transfused cells then circulate until antibodies are produced and immune elimination occurs. A second transfusion with identical foreign cells results in their immediate destruction.

The rapid destruction of large numbers of foreign red cells can lead to serious illness. The severity of transfusion reactions varies from a mild febrile response to death and depends on the amount of incompatible blood transfused. Early recognition of a problem may avert the most severe consequences. The most severe reactions occur when large amounts of incompatible blood are transfused to a sensitized recipient. This results in complement activation and hemolysis of the transfused cells. Large amounts of free hemoglobin escape, resulting in hemoglobinemia and hemoglobinuria. Large numbers of lysed red cells may trigger blood clotting and disseminated intravascular coagulation. Complement activation also results in anaphylatoxin production, mast cell degranulation, and the release of vasoactive molecules and cytokines. These molecules provoke circulatory shock with hypotension, bradycardia, and apnea. The animal may show sympathetic responses such as sweating, salivation, lacrimation, diarrhea, and vomiting. This may be followed by a second stage in which the animal is hypertensive, with cardiac arrhythmia as well as increased heart and respiratory rates.

If a reaction is suspected, the transfusion must be stopped immediately. It is important to maintain urine flow with fluids and a diuretic because accumulation of hemoglobin in the kidney may cause renal tubular destruction. Recovery follows elimination of the foreign red cells.

Transfusion reactions can be prevented by prior testing of the recipient for antibodies against the donor's red cells. The test is called cross-matching. Blood from the donor is centrifuged and the plasma discarded. The red cells are then resuspended in saline and recentrifuged. This washing procedure is repeated (usually three times), and eventually a 2% to 4% suspension of red cells in saline is made. These donor red cells are mixed with recipient serum and then incubated at 37° C for 15 to 30 minutes. If the red cells are lysed or agglutinated by the recipient's serum, then no transfusion should be attempted with those cells. It is occasionally found that the donor's serum may react with the recipient's red cells. This is not of major clinical significance because transfused donor antibodies are rapidly diluted within the recipient. Nevertheless, blood giving such a reaction is best avoided.

26.4 HEMOLYTIC DISEASE OF THE NEWBORN

Female animals may become sensitized to foreign red cells not only by incompatible blood transfusions given for clinical purposes but also by leakage of fetal red cells into their bloodstream through the placenta during

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pregnancy. In sensitized females, these anti-red cell antibodies may then be concentrated in their colostrum. When a newborn animal suckles, these colostral antibodies are absorbed through the intestinal wall and so reach its circulation. These antibodies, directed against the blood group antigens of the newborn, cause rapid destruction of their red cells. The resulting disease is called hemolytic disease of the newborn (HDN) or neonatal isoerythrolysis.

Four conditions must be met for HDN to occur. The young animal must inherit a red cell antigen from its sire that is not present in its mother. The mother must be sensitized to this red cell antigen. The mother's response to this antigen must be boosted repeatedly by transplacental hemorrhage or repeated pregnancies. Finally, a newborn animal must ingest colostrum containing high-titered antibodies to its red cells.

26.5 BLOOD GROUPS, BLOOD TRANSFUSION, AND HEMOLYTIC DISEASE IN DOMESTIC ANIMALS

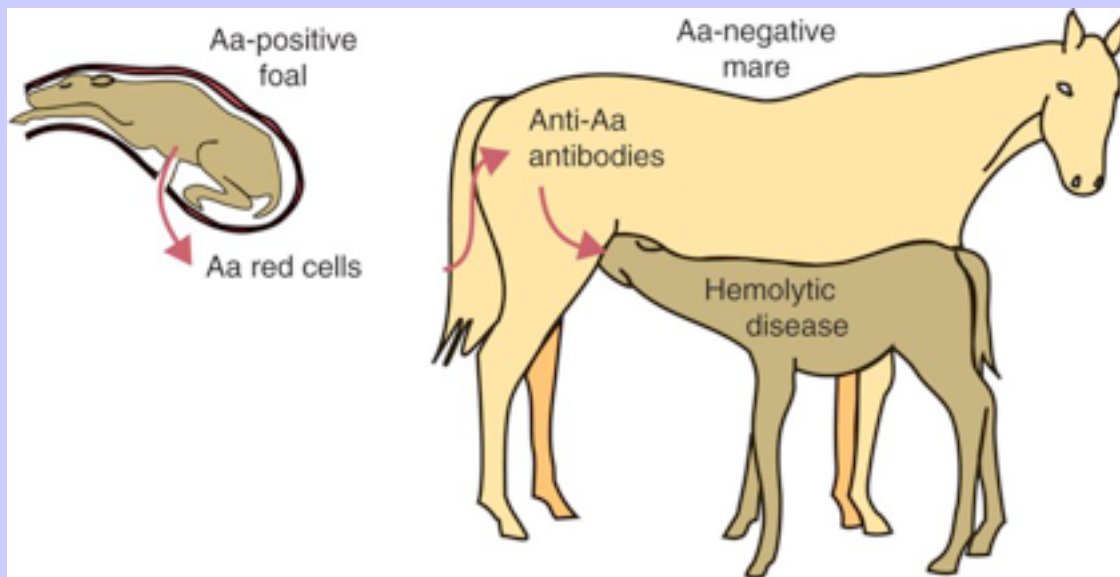
All mammals possess red cell antigens that can interfere with blood transfusions and on occasion cause hemolytic disease in newborn animals ([Table 26-1](#)). Although historically they were named alphabetically in order of their discovery, there is a growing tendency to add the prefix EA (erythrocyte antigen) to reduce confusion with MHC antigens.

26.5.1 Horses

Horses possess seven internationally recognized blood group systems (EAA, EAC, EAD, EAK, EAP, EAQ, and EAU). Some, such as EAC, EAK, and EAU, are simple, one-factor, two-allele, two-phenotype systems. On the other hand, the EAD system is very complex, with at least 25 alleles identified to date. Their major significance lies in the fact that HDN in foals is relatively common ([Figure 26-1](#)). In mules, in which the antigenic differences between dam and sire are great, about 8% to 10% of foals may be affected. In thoroughbreds and standardbreds, the prevalence is considerably less, ranging from 0.05% to 2% of foals. This is in spite of the fact that in up to 14% of pregnancies the mare and the stallion have incompatible red cells.

HDN may occur in foals from mares that have been sensitized by previous blood transfusions or by admin

FIGURE 26-1 The pathogenesis of hemolytic disease of the newborn in foals. In the first stage, fetal lymphocytes leak into the mother's circulation and sensitize her. In the second stage, these antibodies are concentrated in colostrum and are then ingested by the suckling foal. These ingested antibodies enter the foal's circulation and cause red cell destruction.



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istration of vaccines containing equine tissues. Most commonly, however, mares are sensitized by exposure to fetal red cells through repeated pregnancies. The mechanism of this sensitization is unclear, but fetal red cells are assumed to gain access to the maternal circulation as a result of transplacental hemorrhage. Mares have been shown to respond to fetal red cells as early as day 56 after conception. The greatest leakage probably occurs during the last month of pregnancy and during foaling as a result of the breakdown of placental blood vessels.

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Table 26-1 Domestic Animal Blood Groups

Species	Blood Group Systems	Serology
Horse*	A, C, D, K, P, Q, U	Agglutination Hemolytic
Bovine	A, B, C, F, J,† L, M, R,† S, Z, T'	Hemolytic
Sheep	A, B, C, D, M, R†	Hemolytic Agglutination (D only)
Pig*	A,† B, C, D, E, F, G, H, I, J, K, L, M, N, O, P	Agglutination Hemolytic Antiglobulin
Dog	DEA 1.1, 1.2, 3, 4, 5, 6, 7,† 8	Agglutination Hemolytic Antiglobulin
Cat	AB	Agglutination Hemolytic

* In these species there is growing acceptance of the convention to denote red cell antigens with the prefix "EA" (erythrocyte antigen).

† Soluble blood group substances.

Maternal sensitization is usually minimal following a first pregnancy. However, if repeated pregnancies result in exposure to the same red cell antigens, then the maternal response will be boosted. Hemolytic disease is therefore usually only a problem in mares that have had several foals. The most severe form of the disease results from the production of antibodies directed against the Aa antigen of the EAA system. Anti-Qa (EAQ system) produces a less severe disease of slower onset. All in all, 90% of clinical cases are attributable to anti-Aa and anti-Qa, although other minor antigens, such as Pa, Ab, Qc, Ua, Dc, and Db, have also been implicated. Mares that lack Aa and Qa are therefore most likely to produce affected foals. Pregnant mares may also produce antibodies to Ca (EAC system), but these are rarely associated with clinical disease. Indeed preexisting antibodies to Ca may reduce sensitization by Aa. The presence of this anti-Ca in a mare may cause the rapid elimination of any foal red cells that enter its bloodstream and so prevent further sensitization. A red cell antigen that is found in donkeys and mules but not horses causes hemolytic disease in mules. Thus horse mares can readily make antibodies to this donkey antigen.

Antibodies produced by mares do not cross the placenta but reach the foal through the colostrum. Affected foals are therefore born healthy but sicken several hours after suckling. The severity of the disease is determined by the amount of antibody absorbed and by the sensitizing antigen. The earliest signs are weakness and depression. The mucous membranes of affected foals may be pale and may eventually show a distinct jaundice. Some foals sicken by 6 to 8 hours and die from shock so rapidly that they do not have time to develop jaundice. More commonly the disease presents as lethargy and weakness between 12 and 48 hours of age, although it may be delayed for as long as 5 days. Icterus of the mucous membranes and sclera is consistent in foals that survive for

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at least 48 hours. Hemoglobinuria, although uncommon, is pathognomonic in a newborn foal. As a result of anoxia, some foals in the terminal stages of the disease may convulse or become comatose.

Hemolytic disease is readily diagnosed by clinical signs alone. Hematological examination is of little diagnostic use but may be of assistance in indicating appropriate treatment. Definitive diagnosis requires that immunoglobulin be demonstrated on the surface of the red cells of the foal. In the case of anti-Aa or anti-Qa, addition of a source of complement (fresh normal rabbit serum) causes rapid hemolysis. If hemolytic disease is anticipated, the serum of a pregnant mare can be tested for antibodies by an indirect antiglobulin test. Using red cells from horses with a major sensitizing blood group, it is possible to show that the antibody titer increases significantly in the month before parturition, when sensitization is occurring.

A test that may be useful for detecting the presence of antierythrocyte antibodies in colostrum is the jaundiced foal agglutination test. This involves making serial dilutions of colostrum in saline. A drop of anticoagulated foal blood is added to each tube and the tubes are centrifuged so that the red cells form pellets at the bottom. In the presence of antibodies, the cells clump tightly and the pellets remain intact when the tubes are emptied. Unagglutinated red cells, in contrast, flow down the side of the tube. Concentrated colostrum is viscous and tends to induce rouleaux formation that mimics agglutination. However, if the mare's blood is used as a negative control, this can be accounted for. The foal's blood should also be diluted in saline to ensure that the foal has not already absorbed antibodies and that false-positive results are not obtained.

Mildly affected foals (with a packed cell volume [PCV] of 15% to 25% and a red cell count greater than 4×10^6) will continue to nurse. Those with a PCV of less than 10% will stop nursing and become recumbent. Marked icterus is suggestive of HDN in foals, but mild icterus may be seen in septicemia despite the fact that septic foals are not anemic.

The prognosis of uncomplicated hemolytic disease is good provided the condition is diagnosed sufficiently early and the appropriate treatment instituted rapidly. Management of HDN includes prevention of further antibody absorption, adequate nutrition, oxygen therapy, fluid and electrolyte therapy, and maintenance of the acid-base balance. Warmth, adequate hydration, and antimicrobial therapy are also critically important. In acute cases, blood transfusion is necessary. A red cell count less than $3 \times 10^6/\text{mL}$ or a PCV less than 15% warrants a blood transfusion. Transfused equine red cells have a half-life of only 2 to 4 days, so that transfusion is only a temporary life-saving measure. Compatible blood may be difficult to find because of the high prevalence of Aa and Qa in the normal equine population. A donor should not only be Aa or Qa negative but should also lack antibodies to these antigens. Exchange transfusion, though efficient, requires a donor capable of providing at least 5 L of blood as well as a double intravenous catheter and an anesthetized foal. A much simpler technique that avoids many difficulties is transfusion of washed cells from the mare. About 3 to 4 L of blood is collected in sodium citrate and centrifuged, after which the plasma is discarded. The red cells are washed once in saline and transfused slowly into the foal. The blood is usually given in divided doses about 6 hours apart. Milder cases of hemolytic disease may require only careful nursing.

If hemolytic disease is anticipated as a result of either a rising antibody titer or the previous birth of a hemolytic foal, stripping off the mare's colostrum and giving the foal colostrum from another mare may prevent its occurrence. The foal should not be allowed to suckle its mare for 24 to 36 hours. Once suckling is permitted, the foal should only be allowed to take small quantities at first and should be observed carefully for adverse side effects.

Neonatal thrombocytopenia has been recorded in the foal. Immunoglobulins can be identified on the foal's platelets, and antibodies to these platelets can be found in the mare's serum.

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26.5.1.1

Serological Testing

Horse blood groups may be identified by agglutination, hemolytic, and antiglobulin tests. Each blood group system has a preferred test system. The complement used in the hemolytic test comes from rabbits, but it must be absorbed before use to remove any antihorse antibodies.

26.5.2

Cattle

Eleven blood group systems—A, B, C, F, J, L, M, S, T', Z, and R'—have been identified in cattle. Two of these (B and J) are of the greatest importance. The B blood group system is one of the most complex systems known, since it is estimated to contain more than 60 different alleles. These alleles are not inherited independently but in combinations called phenogroups. Because of the complexity of the B system, it is practically impossible to obtain absolutely identical blood from any two unrelated cattle. Indeed, it has been suggested that the complexity of the B system is such that there exist sufficient different antigenic combinations to provide a unique identifying character for each bovine in the world. Naturally, such a system provides an ideal method for the accurate identification of individual animals, and many breed societies use blood grouping to check the identity of registered animals. The C system is also complex, with 10 alleles combining to form about 90 phenogroups.

The J antigen is a lipid found free in body fluids and passively adsorbed onto red cells. It is absent from the red cells of newborn calves but is acquired within the first 6 months of life. J-positive cattle are of two types. Some possess J antigen in high concentration, and this may be detected both on their red cells and in serum. Other animals may have low levels of J antigen in serum, and it is only with great difficulty detected on red cells. (It is probable that a secretor gene controls the expression of J in cattle.) J-negative cattle, lacking the J antigen completely, may possess natural anti-J antibodies, although the level of these antibodies shows a marked seasonal variation, being highest in the summer and fall. Because of the presence of these antibodies, transfusion of J-positive red cells into J-negative recipients may result in a transfusion reaction even in the absence of known previous sensitization.

HDN in calves is rare but has resulted from vaccination against anaplasmosis or babesiosis. Some of these vaccines contain red cells from infected calves. In the case of *Anaplasma* vaccines, for example, the blood from a large number of infected donors is pooled, freeze-dried, and mixed with adjuvant before being administered to cattle. The vaccine against babesiosis consists of fresh, infected calf blood. Both vaccines cause infection and, consequently, the development of immunity in the recipient animals. They may also stimulate the production of antibodies against blood group antigens of the A and F systems. Cows sensitized by these vaccines and then mated with bulls carrying the same blood groups can transmit colostral antibodies to their calves, which may then develop hemolytic disease.

The clinical signs of HDN in calves are related to the amount of colostrum ingested. Calves are usually healthy at birth but begin to show symptoms from 12 hours to 5 days later. In acute cases, death may occur within 24 hours after suckling, with the animals developing respiratory distress and hemoglobinuria. On necropsy these calves have severe pulmonary edema, splenomegaly, and dark kidneys. Less severely affected animals develop anemia and jaundice and may die during the first week of life. The red cells of affected calves have antibodies on their surface (detected by an antiglobulin test) and may sometimes be lysed by the addition of complement in the form of fresh normal rabbit serum. Death is due to disseminated intravascular coagulation as a result of activation of the clotting system by red cell ghosts.

26.5.2.1 Serological Testing

Bovine blood groups are detected by hemolytic tests. Washed red cells are incubated in specific antisera, and rabbit serum is used as a source of complement.

26.5.3 Sheep

The blood groups of sheep resemble those of cattle. Six blood group systems (A, B, C, D, M, and R) are

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FIGURE 26-2 The regulation of expression of R blood group antigens in sheep. The I gene controls expression of the R system.

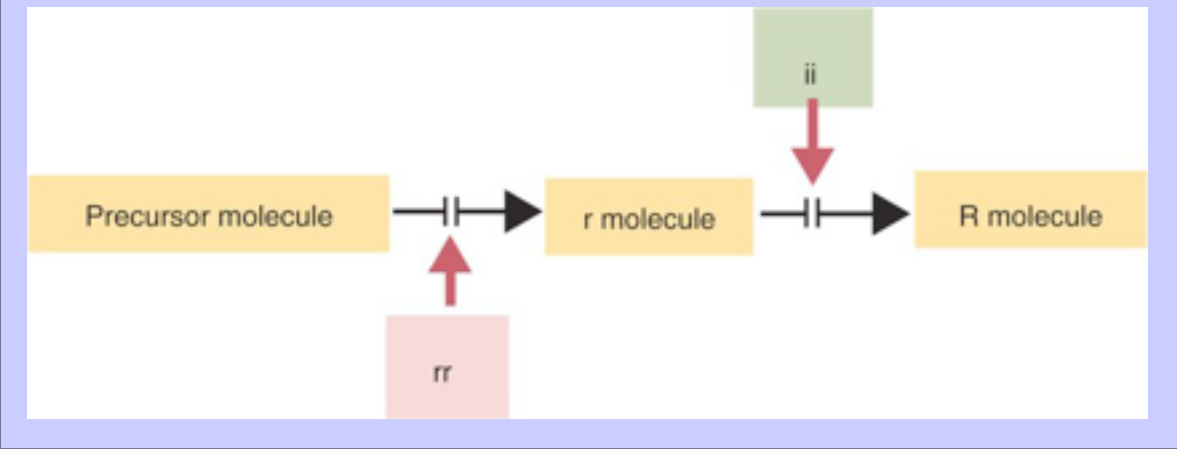
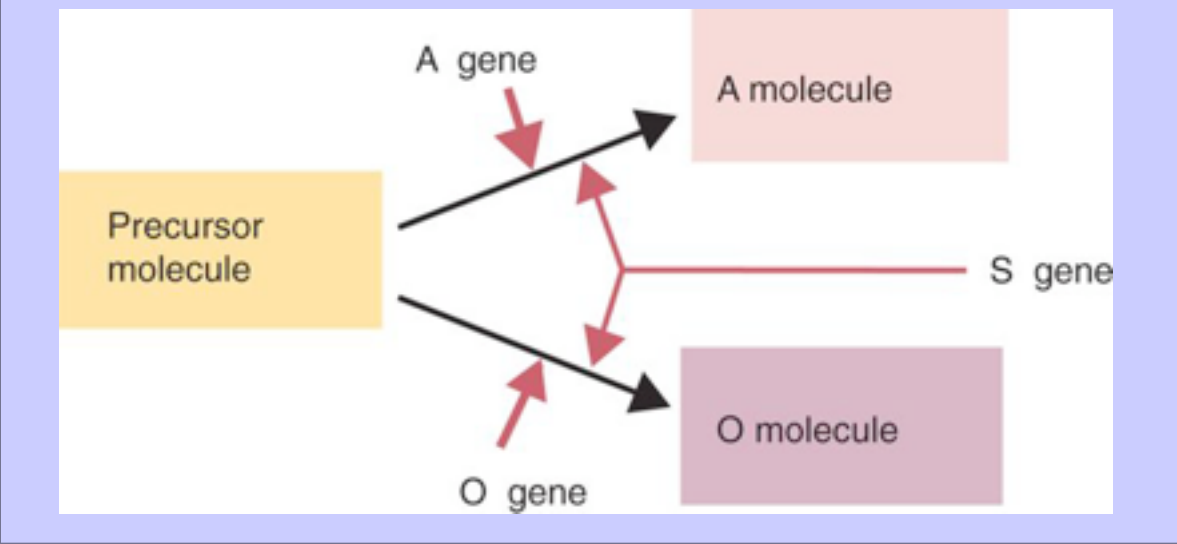


FIGURE 26-3 The production of A or O blood group substances by a pig requires the presence of the S gene. Pigs that lack this gene (ss animals) produce neither of these blood group substances.



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currently recognized. The ovine equivalent of bovine B is also termed B and, like the bovine system, is relatively complex, containing at least 52 different alleles. Sheep also possess an ovine equivalent of the bovine J system, called the R system. Two soluble antigens are found in this system, R and O, coded for by alleles R and r. The production of R and O substances is controlled by a gene called I and its recessive allele i. If a sheep is homozygous for i, it expresses neither R nor O antigens. This interaction between the I/i genes and the R-O system is called an epistatic effect ([Figure 26-2](#)). R and O antigens are soluble antigens found in the serum of II or Ii sheep and are passively adsorbed onto red cells. Natural anti-R antibodies may be found in R-negative sheep. Sheep also fall into two groups according to whether their red cells have high or low potassium levels. This is regulated by the M blood group system. The Mb antigen acts as an inhibitor of potassium transport.

26.5.3.1

Box 26-1 The Inheritance of the A Blood Group System in Pigs

In pigs, the expression of the A blood group is regulated by two gene loci. One locus, the A locus, contains two alleles A and O, of which A is dominant. The other, the S locus, also contains two alleles, S and its recessive allele s. The S locus controls the expression of the A system so that A or O blood groups can only be expressed if the animal carries at least one S gene. Possible genotypes are therefore AA, AO, and OO as well as SS, Ss, and ss.

These may be combined thus:

- Animals that are AASS, AASs, AOSS, or AOSs will have A red cells.
- Animals that are OOSS or OOSs will have O red cells.
- Animals that are AA^{ss}, Aoss, or Ooss will express neither A nor O and so will have “null” red cells.

26.5.3.2

Serological Testing

Sheep blood groups are detected by hemolytic tests. The only exception to this rule is the D system, which is detected by agglutination.

26.5.4

Pigs

Sixteen pig blood group systems have been identified (EAA-EAP). Of these, the most important is the EAA system. The EAA system controls the expression of two antigens, A and O. Their expression is regulated by a gene called S (secretor) with two alleles S and s. In the homozygous recessive state (ss) this gene can prevent the production of the A and O substances ([Figure 26-3](#)). As a result, the amount of these antigens bound to red cells in these animals is reduced to an undetectable level ([Box 26-1](#)). A and O, like J in cattle and R and O in sheep, are not true red cell antigens but soluble molecules found in serum and passively adsorbed onto red cells after birth. Natural anti-A antibodies may occur in A-negative pigs, and transfusion of A-positive blood into such an animal may cause transient collapse and hemoglobinuria.

HDN in piglets formerly occurred as a result of the use of hog cholera vaccine containing pig blood. This vaccine consisted of pooled blood from viremic pigs inactivated with the dye crystal violet. Sensitization of sows by this vaccine led to the occasional occurrence of hemolytic disease of their offspring. There appeared to be a breed predisposition to this disease, which was most commonly seen in the offspring of Essex and Wessex sows. Affected piglets did not necessarily show clinical disease, although their red cells were sensitized by antibody. Other piglets showed rapidly progressive weakness and pallor of mucous membranes preceding death, and those

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animals that survived longest showed hemoglobinuria and jaundice. The severity of the reaction did not appear to be directly related to the anti-red cell antibody titer in the piglet serum. Since the withdrawal of all live hog cholera virus vaccines, the problems associated with their use have disappeared.

True HDN has also been recorded in the pig. The antibodies responsible are usually directed against antigens of the very complex EAE system. In addition to the development of hemolytic anemia in newborn piglets, the presence of antibodies to platelet antigens may cause a thrombocytopenia. This is seen clinically as a bleeding problem on tail docking and a tendency to bruise easily (neonatal purpura). On blood smears, the platelets may be clumped, and antiglobulin testing of them will yield a positive result. Deprivation of colostrum in an attempt to prevent piglets from absorbing anti-red cell antibodies may result in the newborn animals being highly susceptible to infection.

26.5.4.1 Serological Testing

Pig blood groups are detected by agglutination, hemolytic, and antiglobulin tests.

26.5.5 Dogs

In dogs, eight red cell antigens are internationally recognized (DEA 1.1, 1.2, 3, 4, 5, 6, 7, and 8), but five others have been described. (An older nomenclature called them by the traditional alphabetic system, A, Tr, B, C, D, F, J, K, L, M, and N.) The majority of these appear to be inherited as simple Mendelian dominants. Only the DEA 1 antigens are sufficiently antigenic to be of clinical significance. These include the alleles 1.1, 1.2, and 1.3. About 60% of dogs express a DEA 1 antigen. There are no naturally occurring antibodies to DEA 1.1 and 1.2. Antibodies to DEA 7 may occur in 20% to 50% of DEA 7-negative dogs. Antibodies to DEA 1.3, 3, and 5 are found in about 10% of negative dogs, but these are usually of low titer and not of clinical significance. Therefore it is recommended that canine blood donors be negative for DEA 1.1, 1.2, 3, 5, and 7. More than 98% of the canine population is DEA 4 positive. A universal donor would be an animal negative for all the DEA groups except DEA 4. Unless the blood type of the recipient is known, only universal donor blood should be used and a cross-match performed on all recipients, even if universal blood is used. In practice, the most important canine blood type is DEA 1.1. About 33% to 45% of the dog population are DEA 1.1 positive and in general can be considered to be universal recipients. Dogs that are DEA 1.1 negative can also be considered to be universal donors. DEA 1.1-positive blood should never be transfused into a DEA 1.1-negative dog. If so, the recipient will become sensitized to DEA 1.1 blood and high-titered antibodies produced. Subsequent transfusion of positive blood into such an animal could lead to a severe reaction. Similarly, if a negative bitch is sensitized by incompatible transfusions and mated to a positive dog, hemolytic disease may occur in her puppies. Natural HDN in dogs is extremely rare. It occurs when a DEA 1.1-negative breeding bitch is transfused with DEA 1.1-positive blood and subsequently bred to a DEA 1.1-positive male. The puppies develop a hemolytic anemia after 3 to 10 days.

The DEA 7 system (Tr system) is a soluble antigen system antigenically related to the human A, cattle J, sheep R, and pig A systems. Two antigens belong to the system, Tr and O. An epistatic secretor gene controls their expression. Anti-DEA 7 occurs naturally in some DEA 7-negative dogs.

A blood group antigen called Dal has been identified on the basis of antibodies produced in Dalmatians following blood transfusion. Presumably some Dalmatians lack this antigen, which is present in other dog breeds.

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26.5.5.1 Serological Testing

Agglutination at 4° C and hemolytic and antiglobulin tests have all been used for the detection of canine blood groups. The source of complement can be either fresh dog or rabbit serum.

26.5.6 Cats

In cats, only one major blood group system, the AB system, has been reported. The AB antigens are glycolipids. Cats may be A, B, or AB. A is completely dominant over B. About 75% to 95% of cats are A positive, about 5% to 25% are B positive, and less than 1% are AB. However, this distribution differs among countries and among different purebred cat breeds. Thus in the United States more than 99% of domestic short-hair and long-hair cats are type A, whereas in the British short-hair breed only about 40% are type A. Severe transfusion reactions have been described in group B cats that received very small quantities of group A blood since 95% of B cats possess IgM anti-A. (Interestingly, only about 35% of A cats possess anti-B, and it is of the IgG and IgM classes and of much lower titer.) If completely matched blood is transfused into cats, its half-life is about 4 to 5 weeks. If, however, group B blood is transfused into cats of blood group A, its half-life is only a few days. If group A blood is transfused into a cat of blood group B, its half-life is just over 1 hour. It is this very rapid destruction that results in severe clinical reactions. Thus a group B cat given as little as 1 mL of group A blood will go into shock, with hypotension, apnea, and atrioventricular block, within a few minutes. Cross-matching is therefore essential in this species.

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Occasionally, hemolytic transfusion reactions occur between AB blood group-matched cats. These appear to be due to natural antibodies against a blood group antigen called Mik. Its mode of inheritance is undefined.

HDN has been recorded in Persian and related (Himalayan) breeds but is very rare. It occurs in kittens from queens of blood group B bred to sires of blood group A. The queens subsequently develop high-titered anti-A antibodies. Although healthy at birth, these kittens develop severe anemia as a result of intravascular hemolysis. Affected kittens show depression and possibly hemoglobinuria. Necropsy may reveal splenomegaly and jaundice. Antibodies to the sire's and the kitten's red cells are detectable in the queen's serum.

26.5.6.1 Serological Testing

Agglutination and hemolytic tests are used for feline blood typing.

26.5.7 Humans

In humans, HDN is due almost entirely to immunization of the mother against the antigens of the Rhesus (Rh) system (now classified as CD240). The condition is, or should be, of historical interest only because a very simple but effective technique is available for its prevention. This depends on preventing an Rh-negative mother from reacting to the Rh-positive fetal red cells that escape from the placenta into her circulation at birth. Strong human anti-Rh globulin is obtained from male volunteers and given to mothers at risk soon after birth. It acts by specifically inhibiting the B cell response to that antigen (see [Chapter 17](#)). Routine use of this material therefore prevents maternal sensitization, antibody production, and hemolytic disease. The use of a similar system in the domestic mammals is unnecessary because deprivation of colostrum is sufficient to prevent the disease.

26.5.8 Chickens

Chickens have at least 12 different blood group systems with multiple alleles. The red cell B system is also the major histocompatibility system in the chicken. A hemolytic disease may be artificially produced in chicken embryos by vaccinating the hen with cock red cells.

26.6 PARENTAGE TESTING

Under some circumstances it is necessary to confirm the parentage of an animal. One way of doing this is by examining the blood group antigens of an animal and its alleged parents ([Table 26-2](#)). The method is based on the principle that since blood group antigens are inherited, they must be present on the red cells of one or both parents. If a blood group antigen is present in a tested animal but absent from both its putative parents, then parentage must be reassigned. Similarly, if one parent is homozygous for a specific blood group antigen, this antigen must appear in the offspring. However, it must be recognized that blood typing procedures can only exclude, never prove, parentage.

26.7 HEMOPHAGOCYTIC SYNDROME

Hemophagocytic syndrome is a benign proliferative disorder of activated macrophages associated with multiple cytopenias in the blood. These cytopenias result from hemophagocytosis and probably reflect excessive phagocytic activity by macrophages. The syndrome has been described in humans, dogs, and cats. In humans it may be either inherited or acquired. In dogs, the syndrome has been reported as secondary to infectious, neoplastic, or immune-mediated diseases. Diagnostic criteria include the presence of pancytopenia or bicytopenia and the presence of greater than 2% hemophagocytic macrophages in a bone marrow aspirate. Most of these dogs have an underlying disease condition. Thus about a third of canine cases are associated with immune-mediated diseases such as lupus or immune-mediated thrombocytopenia. These animals are commonly anemic, neutropenic, and thrombocytopenic and it may be argued that autoantibodies opsonized the blood cells leading to their phagocytosis. Other affected dogs suffer from infectious diseases such as pyometra, pleuritis, ehrlichiosis, blastomycosis, or lyme disease. In some cases affected dogs recover once their underlying infection is treated. The disease is also associated with some neoplastic diseases such as malignant lymphoma or myelodysplastic syndrome.

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Table 26-2 The Use of Blood Groups to Assign Paternity

	Blood Group				
	1.1	1.2	6	7	8
Sire 1 ?	+	+	-	+	-
Sire 2 ?	+	+	-	-	+
Dam	-	-	+	+	-
Puppy 1	+	+	-	-	-
2	+	+	-	+	-
3	-	-	-	+	+
4	-	-	+	+	-
Courtesy Dr. D Colling.					

* This puppy possesses DEA 8, which could not have come from sire 1 or its dam. Sire 1 could not have sired this litter.

Cases of canine hemophagocytic syndrome also occur in the absence of any obvious associated disease. Affected dogs are anemic, neutropenic, thrombocytopenic, febrile, anorexic, and lethargic. In humans this syndrome may result from an NK cell deficiency or as a result of excessive macrophage activation resulting from oversecretion of Th1 cytokines.

26.8 TYPE II HYPERSENSITIVITY REACTIONS TO DRUGS

Red cells may be destroyed in drug hypersensitivities by three mechanisms. First, the drug and antibody may combine directly and activate complement, and red cells will be destroyed in a bystander effect as activated complement components bind to nearby cells.

Second, some drugs may bind firmly to cells, especially those in the blood. For example, penicillin, quinine, L-dopa, aminosalicic acid, and phenacetin may adsorb onto the surface of red cells. Since these cells are then modified, they may be recognized as foreign and eliminated by an immune response, resulting in hemolytic anemia. Penicillin-induced hemolytic anemia is not uncommon in horses. These conditions can be suspected based on recent treatment with penicillin and improvement when its use is discontinued. It may also be possible to detect antibodies against penicillin or penicillin-coated red cells in these animals. Sulfonamides, phenylbutazone, aminopyrine, phenothiazine, and possibly chloramphenicol may cause agranulocytosis by binding to granulocytes, and phenylbutazone, quinine, chloramphenicol, and sulfonamides may provoke thrombocytopenia. If the cells from animals experiencing these reactions are examined using a direct antiglobulin test, antibody may be demonstrated on their surface. If these antibodies are eluted, they can be directed not against the blood cells but against the offending drug.

Third, drugs such as the cephalosporins may modify red cell membranes in such a way that the cells passively adsorb antibodies and then are removed by phagocytic cells.

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26.9 TYPE II HYPERSENSITIVITY IN INFECTIOUS DISEASES

Just as drugs can be adsorbed onto red cells and render them immunologically foreign, so also can bacterial antigens such as the lipopolysaccharides, viruses such as equine infectious anemia virus and Aleutian disease virus, rickettsia such as *Anaplasma*, and protozoa such as the trypanosomes and *Babesia*. These altered red cells are regarded as foreign and are either lysed by antibody and complement or phagocytosed by mononuclear phagocytes. Clinically severe anemia is, therefore, characteristic of all these infections.

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²⁷ CHAPTER 27 Immune Complexes and Type III Hypersensitivity

^{27.1} KEY POINTS

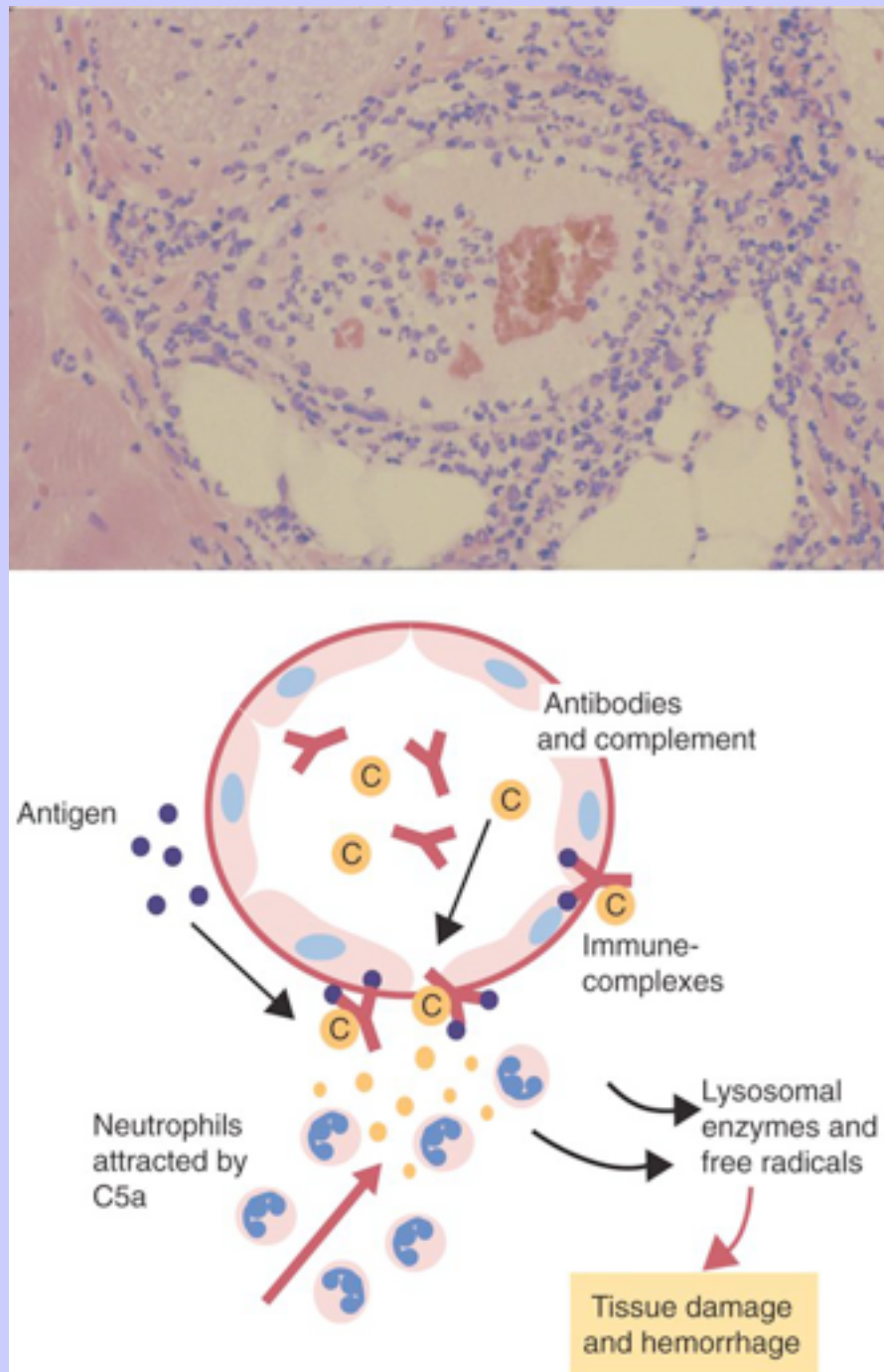
- When antigens and antibodies combine, they form immune complexes. Immune complexes can cause severe inflammation when deposited in large amounts in tissues. This type of inflammation is classified as type III hypersensitivity.
- Local deposition of immune complexes in the lungs following inhalation of antigenic dusts causes hypersensitivity pneumonitis.
- Immune complexes formed in the bloodstream are deposited in the glomeruli of the kidney and cause membranoproliferative glomerulonephritis.
- Type III hypersensitivity is a feature of many viral diseases, especially if the virus is not neutralized by antibodies and, as a result, large amounts of immune complexes are generated.

Acute inflammation can be triggered by the presence of immune complexes in tissues. Immune complexes formed by the combination of antibodies with antigen activate complement. When these immune complexes are deposited in tissues, the activated complement generates chemotactic peptides that attract neutrophils. The accumulated neutrophils may then release oxidants and enzymes, causing acute inflammation and tissue destruction. Lesions generated in this way are classified as type III or immune complex-mediated hypersensitivity reactions.

^{27.2} CLASSIFICATION OF TYPE III HYPERSENSITIVITY REACTIONS

The severity and significance of type III hypersensitivity reactions depend, as might be expected, on the amount and site of deposition of immune complexes. Two major forms of reaction are recognized. One form includes local reactions that occur when immune complexes form within tissues. The second form results when large quantities of immune complexes form within the bloodstream. This can occur, for example, when an antigen is administered intravenously to an immune recipient. Immune complexes generated in

FIGURE 27-1 The mechanisms of an Arthus reaction, as well as a histological section of an Arthus reaction in the skin of a cat 6 hours after intradermal inoculation of chicken red blood cells. (Courtesy Dr. A. Kier.)



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the bloodstream are deposited in glomeruli in the kidney, and the development of glomerular lesions (glomerulonephritis) is characteristic of this type of hypersensitivity. If the complexes bind to blood cells, anemia, leukopenia, or thrombocytopenia may also result. Complexes may also be deposited in blood vessel walls to cause a vasculitis or in joints to cause arthritis.

It might reasonably be pointed out that the combination of an antigen with antibody always produces immune complexes. However, the occurrence of clinically significant type III hypersensitivity reactions results from the formation of excessive amounts of these immune complexes. For example, several grams of an antigen are needed to sensitize an animal, such as a rabbit, in order to produce experimental type III reactions. Minor immune complex-mediated lesions probably develop relatively frequently following an immune response to many antigens without causing clinically significant disease.

27.3 LOCAL TYPE III HYPERSENSITIVITY REACTIONS

If an antigen is injected subcutaneously into an animal that already has precipitating antibodies in its bloodstream, acute inflammation will develop at the injection site within several hours. This is called an Arthus reaction after the scientist who first described it. It starts as a red, edematous swelling; eventually local hemorrhage and thrombosis occur, which, if severe, culminate in local tissue destruction.

The first histological changes observed following antigen injection are neutrophil adherence to vascular endothelium followed by their emigration into the tissues. By 6 to 8 hours, when the reaction has reached its greatest intensity, the injection site is densely infiltrated by large numbers of these cells ([Figure 27-1](#)). As the reaction progresses, destruction of blood vessel walls results in hemorrhage and edema, platelet aggregation, and thrombosis. By 8 hours, mononuclear cells appear in the lesion, and by 24 hours or later, depending on the amount of antigen injected, they become the predominant cell type. Eosinophils are not a significant feature of this type of hypersensitivity.

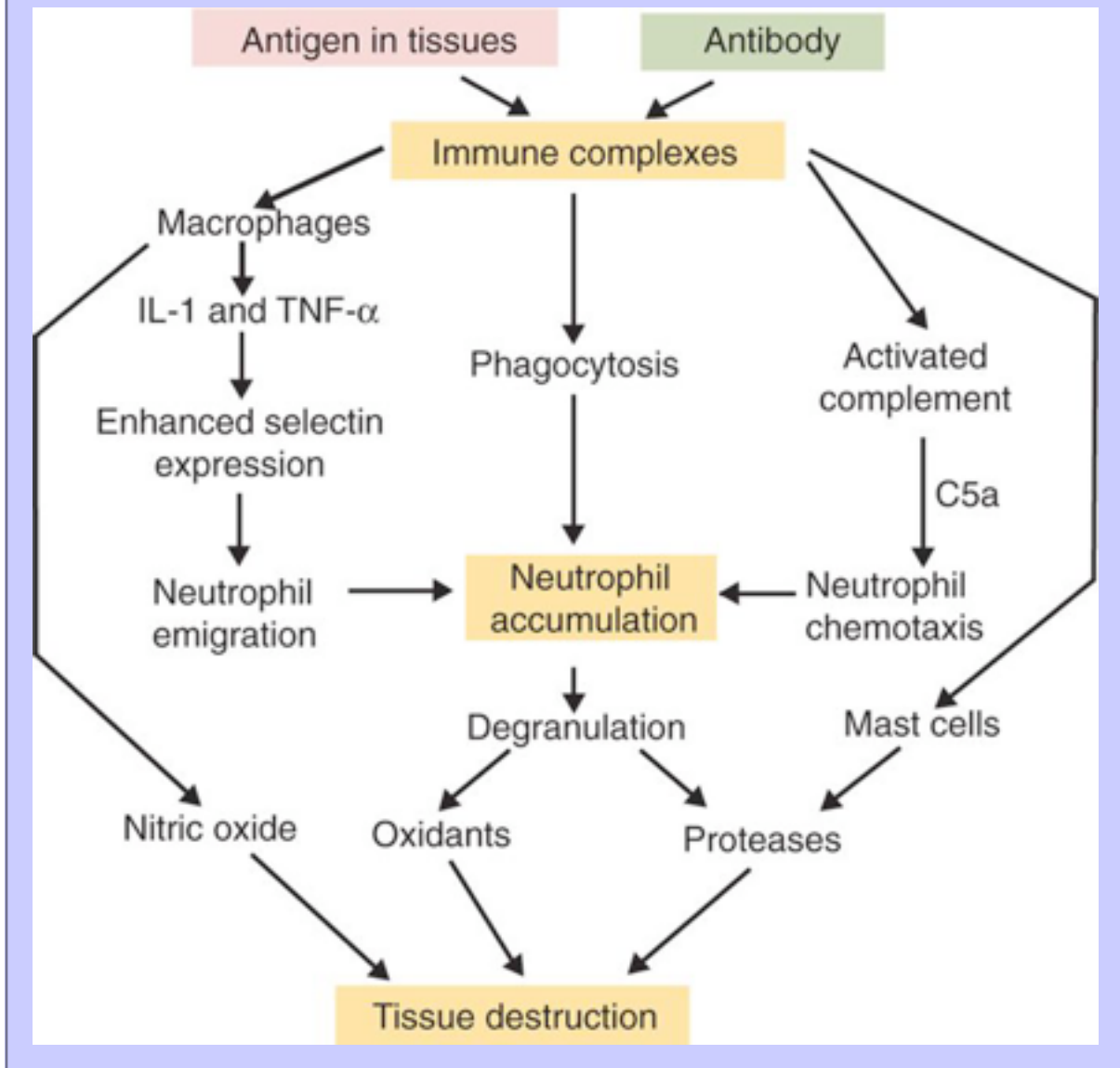
The fate of the injected antigen can be followed using a direct fluorescent antibody test. The antigen first diffuses away from the injection site through tissue fluid. When small blood vessels are encountered, the antigen diffuses into the vessel walls, where it encounters circulating antibodies. Provided the antibodies involved are both precipitating and complement activating (and are therefore usually immunoglobulin G [IgG]), immune complexes form and are deposited between and beneath vascular endothelial cells.

The immune complexes act on two major cell types: mast cells and neutrophils. The relative contribution of each varies among tissues.

Immune complexes formed in tissues must be removed. This is done by binding to Fc and complement receptors on cells. The most widespread of these Fc receptors is FcγRIIa on sentinel cells. Immune complexes binding to these receptors on macrophages stimulate production of nitric oxide, leukotrienes, prostaglandins, cytokines, and chemokines. Immune complexes also bind to mast cells through FcγRIII. This binding triggers the mast cells to release their vasoactive molecules. Among the molecules released by mast cells are neutrophil chemotactic factors and proteases that activate complement, cytokines, kinins, and lipid mediators. All these mediators promote inflammation by acting on vascular endothelium and stimulating neutrophil adherence and emigration.

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FIGURE 27-2 Some of the mechanisms involved in the pathogenesis of the Arthus reaction.



The immune complexes activate complement to generate the chemotactic peptide C5a (Figure 27-2). The neutrophils, attracted by C5a as well as mast cell–derived chemotactic factors, emigrate from the blood vessels, adhering to immune complexes and promptly phagocytosing them. Eventually the immune complexes are eliminated. During this process, however, proteases and oxidants are released into the tissues. When neutrophils attempt to ingest immune complexes attached to a structure such as a basement membrane, they secrete their granule contents directly into the surrounding tissues. Neutrophil proteases disrupt collagen fibers and destroy ground substances, basement membranes, and elastic tissue. Normally tissues contain antiproteases that inhibit neutrophil enzymes. However, neutrophils can subvert these inhibitors by secreting OCI^- . The OCI^- destroys the inhibitors and allows tissue destruction to proceed.

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Although it has long been assumed that immunoglobulin molecules do not themselves damage antigens, recent evidence has shown that they can kill microorganisms and cause tissue damage. When provided with singlet oxygen from phagocytic neutrophils, antibodies catalyze the production of oxidants including ozone. This ozone not only kills bacteria but also kills nearby cells. Biopsies from Arthus reactions contain detectable amounts of ozone.

Neutrophil proteases also act on C5 to generate C5a, which stimulates neutrophil degranulation and enzyme release and so promotes further neutrophil accumulation and degranulation. Other enzymes released by neutrophils make mast cells degranulate or generate kinins. As a result of all this, inflammation and destruction of tissues (especially of blood vessel walls) result in the development of the edema, vasculitis, and hemorrhage characteristic of an Arthus reaction.

Although the classical direct Arthus reaction is produced by local administration of an antigen to hyperimmunized animals, any technique that deposits immune complexes in tissues will stimulate a similar response. A reversed Arthus reaction can therefore be produced if antibodies are administered intradermally to an animal with a high level of circulating antigen. Injected, preformed immune complexes, particularly those containing a moderate excess of an antigen, will provoke a similar reaction, although, as might be anticipated, there is less involvement of blood vessel walls and the reaction is less severe. A passive Arthus reaction can be produced by giving antibody intravenously to a nonsensitized animal followed by an intradermal injection of an antigen, and real enthusiasts can produce a reversed passive Arthus reaction by giving antibody intradermally followed by intravenous antigen.

Although it is unusual for pure hypersensitivity reactions of only a single type to occur under natural conditions, there are some diseases in the domestic animals in which type III reactions play a major role. The classical Arthus reaction is usually produced in the skin, since that is the most convenient site at which to inject the antigen. However, local type III reactions can occur in many tissues, with the precise site depending on the location of the antigen.

27.3.1 Blue Eye

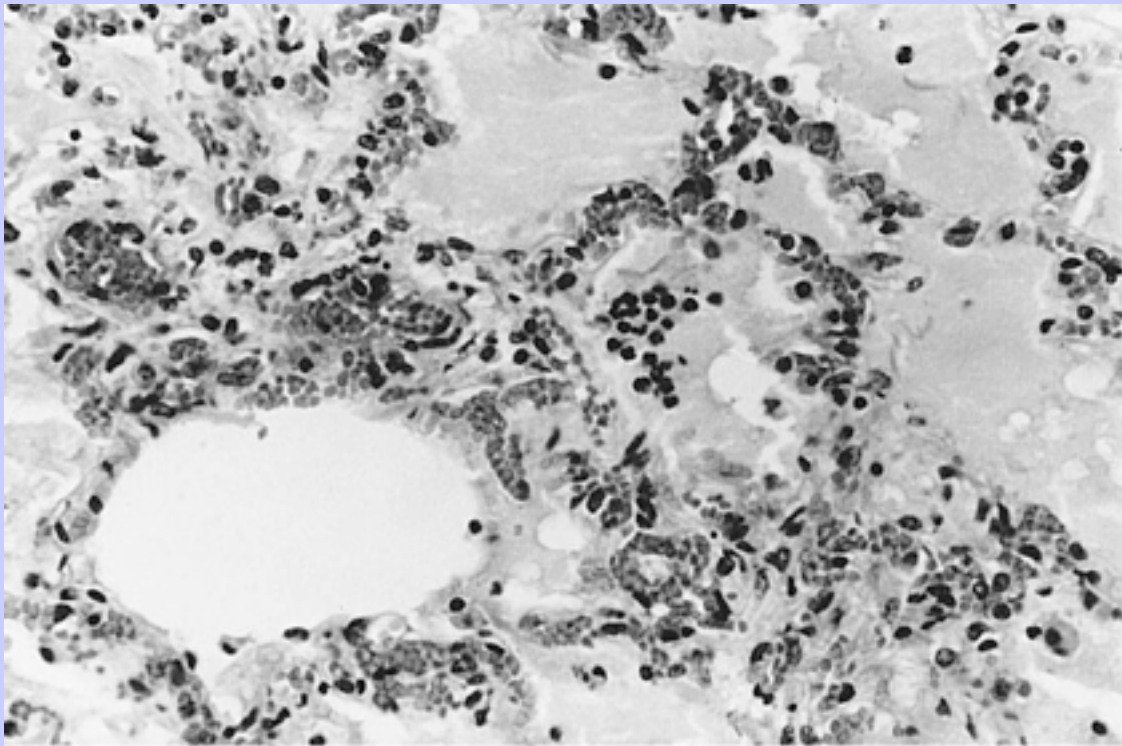
Blue eye is a condition seen in a small proportion of dogs that have been either infected or vaccinated with live canine adenovirus type 1 (see [Chapter 23](#), [Figures 23-7](#) and [23-8](#)). The lesion in blue eye is an anterior uveitis leading to corneal edema and opacity. The cornea is infiltrated by neutrophils, and virus-antibody complexes can be detected in the lesion. Blue eye develops about 1 to 3 weeks after the onset of infection and usually resolves spontaneously as virus is eliminated.

27.3.2 Hypersensitivity Pneumonitis

Type III hypersensitivity reactions may occur in the lungs when sensitized animals inhale antigens. For example, cattle housed during the winter are usually exposed to dust from hay. Normally, these dust particles are relatively large and are deposited in the upper respiratory tract, trapped in mucus, and eliminated. If, however, hay is stored when damp, bacterial growth and metabolism will result in heating. As a result of

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FIGURE 27-3 A histological section of the lung from a cow that died suddenly 24 hours after being fed moldy hay. The alveoli are full of fluid and the alveolar walls are thickened and inflamed. This acute alveolitis is probably due to a hypersensitivity reaction to inhaled actinomycete spores. Original magnification $\times 400$. (Courtesy Dr. B.N. Wilkie.)



this warmth, thermophilic actinomycetes will grow. One of the most important of these thermophilic actinomycetes is *Saccharopolyspora rectivirgula* (*Micropolyspora faeni*), an organism that produces large numbers of very small spores ($1\ \mu\text{m}$ in diameter). On inhalation, these spores can penetrate to the alveoli (see [Chapter 19, Figure 19-4](#)). If cattle are fed moldy hay for long periods, constant inhalation of *S. rectivirgula* spores will result in sensitization and in the development of high-titered precipitating antibodies to *S. rectivirgula* antigens in serum. Eventually inhaled spore antigens will encounter antibodies within the alveolar walls, and the resulting immune complexes and complement activation will cause a pneumonia (or pneumonitis), the basis of which is a type III hypersensitivity reaction.

The lesions of this hypersensitivity pneumonitis consist of an acute alveolitis together with vasculitis and exudation of fluid into the alveolar spaces ([Figure 27-3](#)). The alveolar septa may be thickened, and the entire lesion is infiltrated with inflammatory cells. Since many of these cells are eosinophils and lymphocytes, it is obvious that the reaction is not a pure type III reaction. Nevertheless, examination of the lungs of affected cattle by immunofluorescence demonstrates deposits of immunoglobulin, complement, and antigen. In animals inhaling low levels of an antigen over a long period, proliferative bronchiolitis and fibrosis may be observed.

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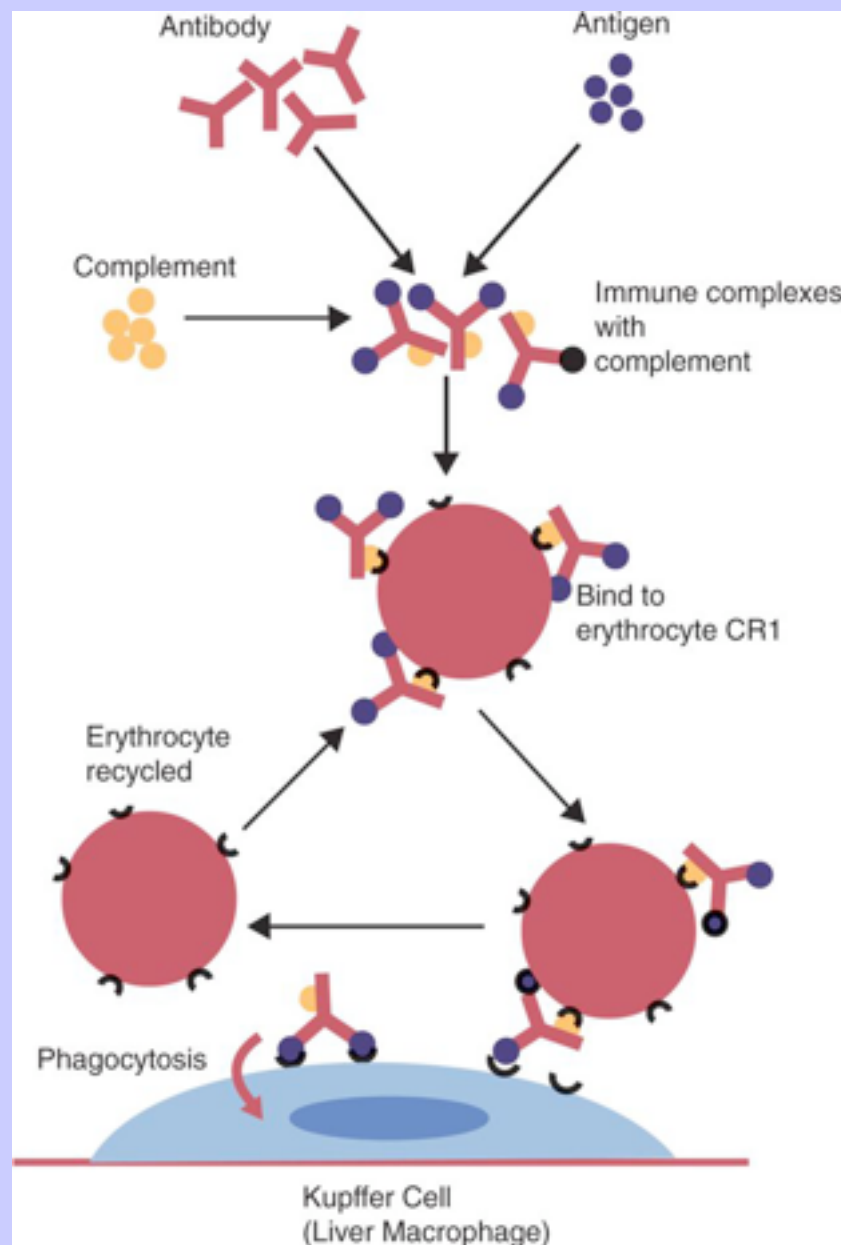
Clinically, hypersensitivity pneumonitis presents as a pneumonia occurring between 5 and 10 hours after acute exposure to grossly moldy hay. The animal may have difficulty breathing and develop a severe cough. In chronically affected animals, the dyspnea may be continuous. The most effective method of managing this condition is by removing the source of the antigen. Administration of steroids may be beneficial.

A hypersensitivity pneumonitis also occurs in farmers chronically exposed to *S. rectivirgula* spores from moldy hay and is called farmer's lung. Many other syndromes in humans have an identical pathogenesis and are usually named after the source of the offending antigen. Thus pigeon breeder's lung arises following exposure to the dust from pigeon feces, mushroom grower's disease is due to hypersensitivity to inhaled spores from actinomycetes in the soil used for growing mushrooms, and librarian's lung results from inhalation of dusts from old books. Hay sickness is a hypersensitivity pneumonitis seen in horses in Iceland that is probably an equine equivalent of farmer's lung.

Two forms of chronic respiratory disease occur in horses. Recurrent airway obstruction (RAO) is seen in older horses, and inflammatory airway disease (IAD) is seen in horses of any age. Both are forms of chronic bronchiolitis associated with mold and other allergen exposure in dusty stable air. RAO occurs most obviously in horses that inhale large amounts of organic dusts. It includes obstructive pulmonary disease seen in stabled horses and summer pasture-associated obstructive pulmonary disease. Characteristically horses with RAO suffer from respiratory difficulty (heaves) even while at rest. Horses with IAD, in contrast, show poor performance, exercise intolerance, and coughing. The disease is not associated with obvious infections. Horses with these syndromes may show positive skin reactions to intradermal inoculation of actinomycete and fungal extracts (such as *Rhizopus nigricans*, *Candida albicans*, *S. rectivirgula*, or *Geotrichum deliquescens*). They may also respond to aerosol challenge with extracts of these organisms by developing respiratory distress. Clinical signs may resolve on removal of the moldy hay and reappear on reexposure. However, there is little correlation between skin test results and severity of disease. Affected animals usually have large numbers of neutrophils or eosinophils in their small bronchioles, and high titers of antibodies to equine influenza in their bronchial secretions. The significance of the latter is unclear. However, high levels of the chemokine CXCL8 (interleukin-8 [IL-8]) are found in the bronchoalveolar washings of affected animals. Affected horses may react more strongly than normal to histamine. Removal of clinically affected horses to air-conditioned stalls results in improvement of the disease, but this is reversed if the horses are returned to dusty stables. It has been suggested that continuous prolonged activation of bronchoalveolar macrophages by dust particles and air-borne endotoxins leads to excessive production of neutrophil chemotactic chemokines such as CXCL8 and CXCL2. These neutrophils then

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FIGURE 27-4 In primates, immune complexes are removed by binding to complement receptors on red blood cells. They are then carried to the liver, where they are transferred to Kupffer cells for phagocytosis. In the absence of complement components, significant accumulation of immune complexes occurs in tissues. In other mammals, immune complexes bind to receptors on platelets.



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cause lung damage as a result of secretion of proteases, peroxidases, and oxidants. IAD affects up to 30% of young horses in training. Although commonly linked to bacterial or viral infections, in many cases no infectious agent can be isolated. Affected animals have airway inflammation associated with a neutrophil infiltration, but occasionally eosinophils and mast cells may be increased.

27.3.3 Staphylococcal Hypersensitivity

Staphylococcal hypersensitivity is a pruritic pustular dermatitis of dogs. Skin testing with staphylococcal antigens suggests that types I, III, and IV hypersensitivity may be involved. The histological findings of neutrophilic dermal vasculitis suggest that the type III reaction may predominate in some cases.

27.4 GENERALIZED TYPE III HYPERSENSITIVITY REACTIONS

If an antigen is administered intravenously to animals with a high level of circulating antibodies, immune complexes form in the bloodstream. These immune complexes are normally removed by binding to either erythrocytes or platelets ([Figure 27-4](#)), or, if very large, they are removed by the mononuclear phagocyte system. However, if complexes are produced in excessive amounts, they may be deposited in the walls of blood vessels, especially medium-sized arteries, and in vessels where there is a physiological outflow of fluid such as glomeruli, synovia, and the choroid plexus ([Figure 27-5](#)). An excellent example of this type of hypersensitivity is serum sickness.

27.4.1 Serum Sickness

Many years ago, when the use of antisera for passive immunization was in its infancy, it was observed that human patients who had received a very large dose of equine antitetanus serum developed a characteristic reaction about 10 days later. This reaction, called serum sickness, consisted of a generalized vasculitis with erythema, edema, and urticaria of the skin, neutropenia, lymph node enlargement, joint swelling, and proteinuria. The reaction was usually of short duration and subsided within a few days. A similar reaction can be produced experimentally in rabbits by administration of a large intravenous dose of antigen. The development of lesions coincides with the formation of large amounts of immune complexes in the circulation as a result of the immune response to circulating antigens ([Figure 27-6](#)). The experimental disease may be acute if it is caused by a single, large injection of an antigen or chronic, if caused by multiple small injections. In either case, animals develop a glomerulonephritis ([Figure 27-7](#)) and an arteritis.

27.4.2 Glomerulonephritis

When immune complexes are deposited in the glomeruli, they cause basement membrane thickening and stimulate glomerular cells to proliferate. Any or all of the three glomerular cell populations—epithelial cells, endothelial cells, and mesangial cells—can proliferate. The lesion is therefore called membranoproliferative glomerulonephritis (MPGN). If immune complexes are deposited only in the mesangium, mesangial cell proliferation will result in a mesangioproliferative glomerulonephritis. MPGN lesions are classified into three major types based on their histopathology ([Figure 27-8](#)).

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FIGURE 27-5 The mechanisms involved in the pathogenesis of acute serum sickness.

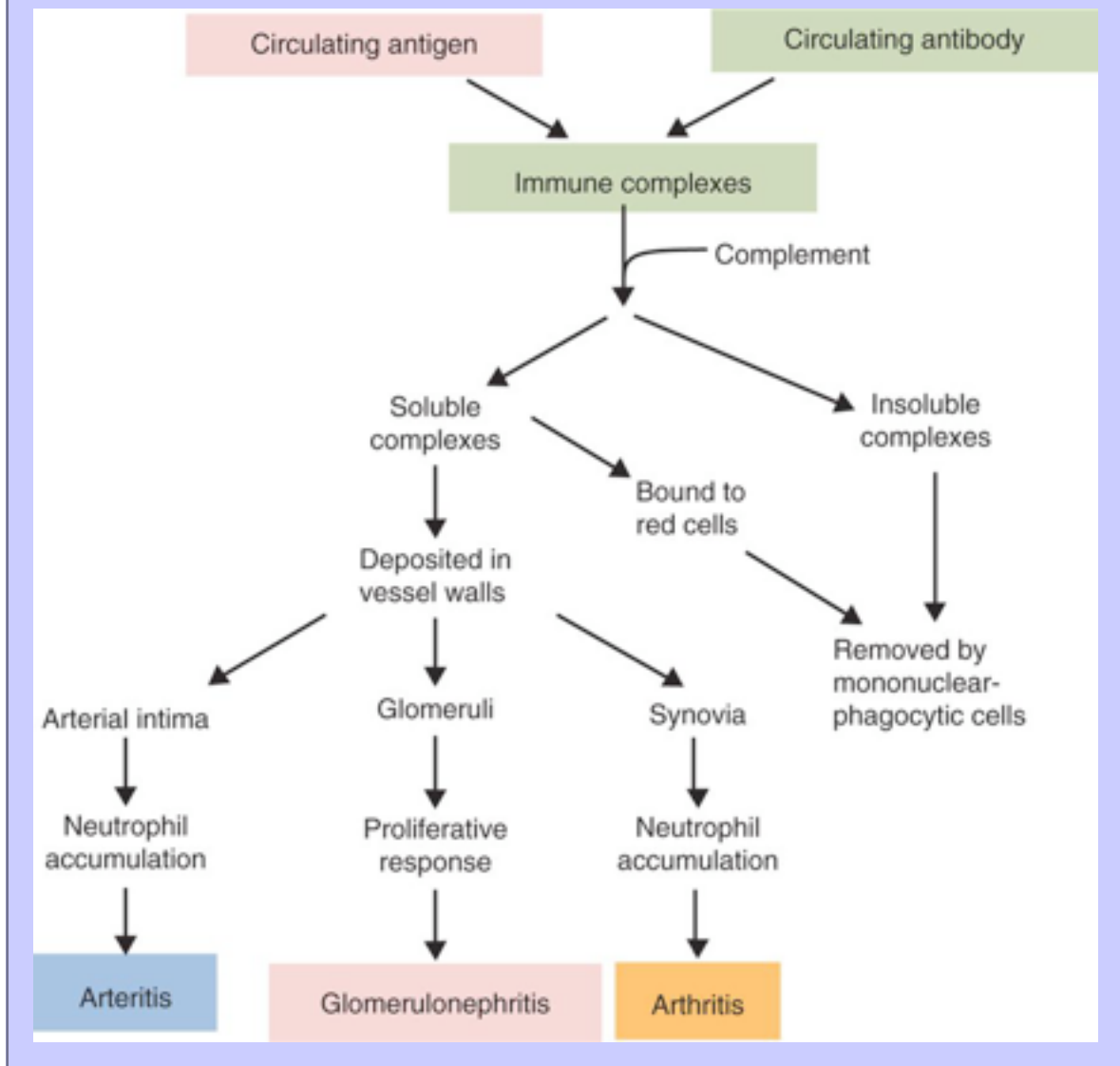
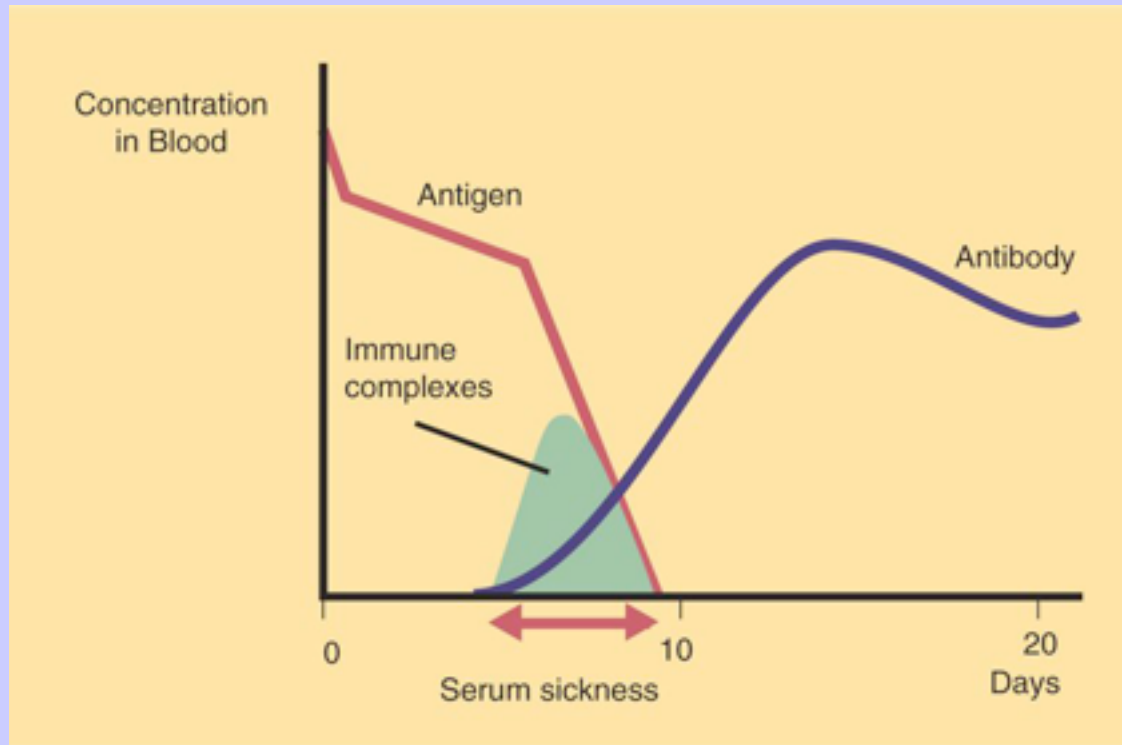


FIGURE 27-6 The time course of acute serum sickness. The appearance of the disease coincides with the generation of immune complexes in the bloodstream.



27.4.2.1

Type I Membranoproliferative Glomerulonephritis

Type I MPGN is caused by immune complex deposition in glomerular vessels. These complexes usually penetrate the vascular endothelium but not the basement membrane and are therefore trapped on the endothelial side, where they stimulate endothelial cell swelling and proliferation ([Figure 27-9](#)). If an animal is given repeated injections of small doses of an antigen over a long period, continued damage to the glomerular cells by immune complexes leads to production of transforming growth factor- β (TGF- β). This cytokine stimulates nearby cells to produce fibronectin, collagen, and proteoglycans. This results in a thickening of the basement membrane to form the so-called wire

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FIGURE 27-7 The structure of a typical glomerulus. Immune complexes may be deposited on either side of, or within, the glomerular basement membrane.

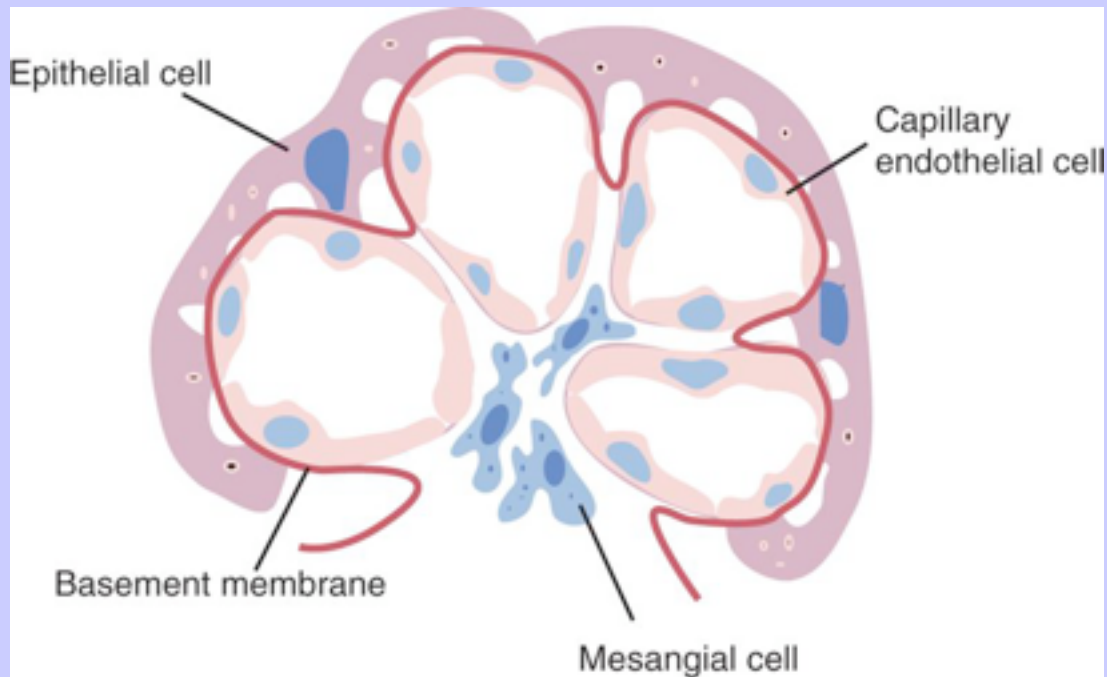
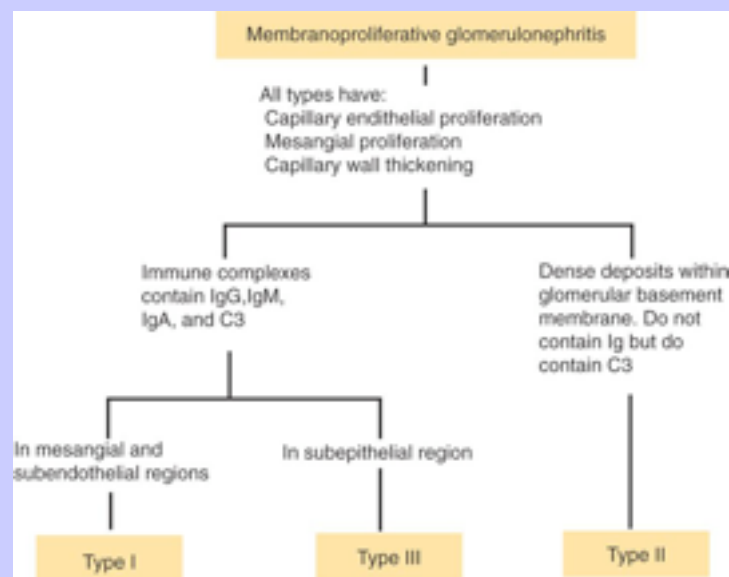


FIGURE 27-8 A classification of different forms of membranoproliferative glomerulonephritis.



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loop lesion (also called a membranous glomerulonephritis). Alternatively, the immune complexes may be deposited in the mesangial region of glomeruli. Mesangial cells are modified smooth muscle cells. As such they can release cytokines and prostaglandins and take up immune complexes. They respond to the immune complexes by proliferation and release of IL-6 and TGF- β . The IL-6 stimulates autocrine growth of the mesangial cells. The TGF- β stimulates production of extracellular matrix. This mesangioproliferative glomerulonephritis eventually interferes with glomerular function. By immunofluorescence it can be shown that lumpy aggregates of immune complexes are deposited in capillary walls and on the epithelial side of the glomerular basement membrane ([Figure 27-10](#)).

27.4.2.2

Type II Membranoproliferative Glomerulonephritis

Type II MPGN (or dense deposit disease) is similar to the type I disease in that there is endothelial and mesangial proliferation. However, it is characterized by the presence of homogeneous, dense deposits within the glomerular basement membrane (in the lamina densa) rather than on its surface (see [Chapter 5, Figure 5-17](#)). The deposits may contain C3 but not immunoglobulin. Type II MPGN results from uncontrolled complement activation and is seen in factor H deficiency in pigs (see [Chapter 5](#)).

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27.4.2.3

Type III Membranoproliferative Glomerulonephritis

Type III MPGN is a variant of type I MPGN. It differs from typical type I disease by the presence of immune complexes on both the endothelial and epithelial sides of the basement membrane. It is believed that very small immune complexes penetrate the basement membrane and are deposited where they stimulate epithelial cell swelling and proliferation. If excessive, these proliferating cells may fill the glomerular space to form epithelial crescents. A single case of unknown cause has been described in a cat.

27.5

CLINICAL FEATURES OF GLOMERULONEPHRITIS

Type I MPGN develops when prolonged antigenemia persists in the presence of antibodies. It is therefore characteristic of chronic viral diseases such as equine infectious anemia, infectious canine hepatitis, Aleutian disease of mink, and African swine fever; parasitic diseases such as leishmaniasis; and chronic bacterial diseases such as Lyme disease and ehrlichiosis ([Table 27-1](#)). Clinically it should be suspected in an animal with proteinuria without evidence of infection although definitive diagnosis requires a renal biopsy and histological evaluation. Type I MPGN has also been reported in dogs with pyometra, chronic pneumonia, distemper encephalitis, acute pancreatic necrosis, and bacterial endocarditis. In animals with tumors, large amounts of antigen may be shed into the bloodstream and give rise to a type I MPGN. This is, for example, a feature of feline leukemia. It has also been reported in animals with lymphosarcoma, osteosarcoma, and mastocytoma. Circulating immune complexes and renal lesions have been found in dogs with systemic lupus erythematosus (see [Chapter 33](#)), discoid lupus, generalized demodicosis, and recurrent staphylococcal pyoderma. Some cases may be due to deficiencies of complement components. As a result of these deficiencies, removal

FIGURE 27-9 The pathogenesis of different forms of immune complex-mediated glomerulonephritis. Remember, however, that more than one type of lesion may be present in an animal at the same time.

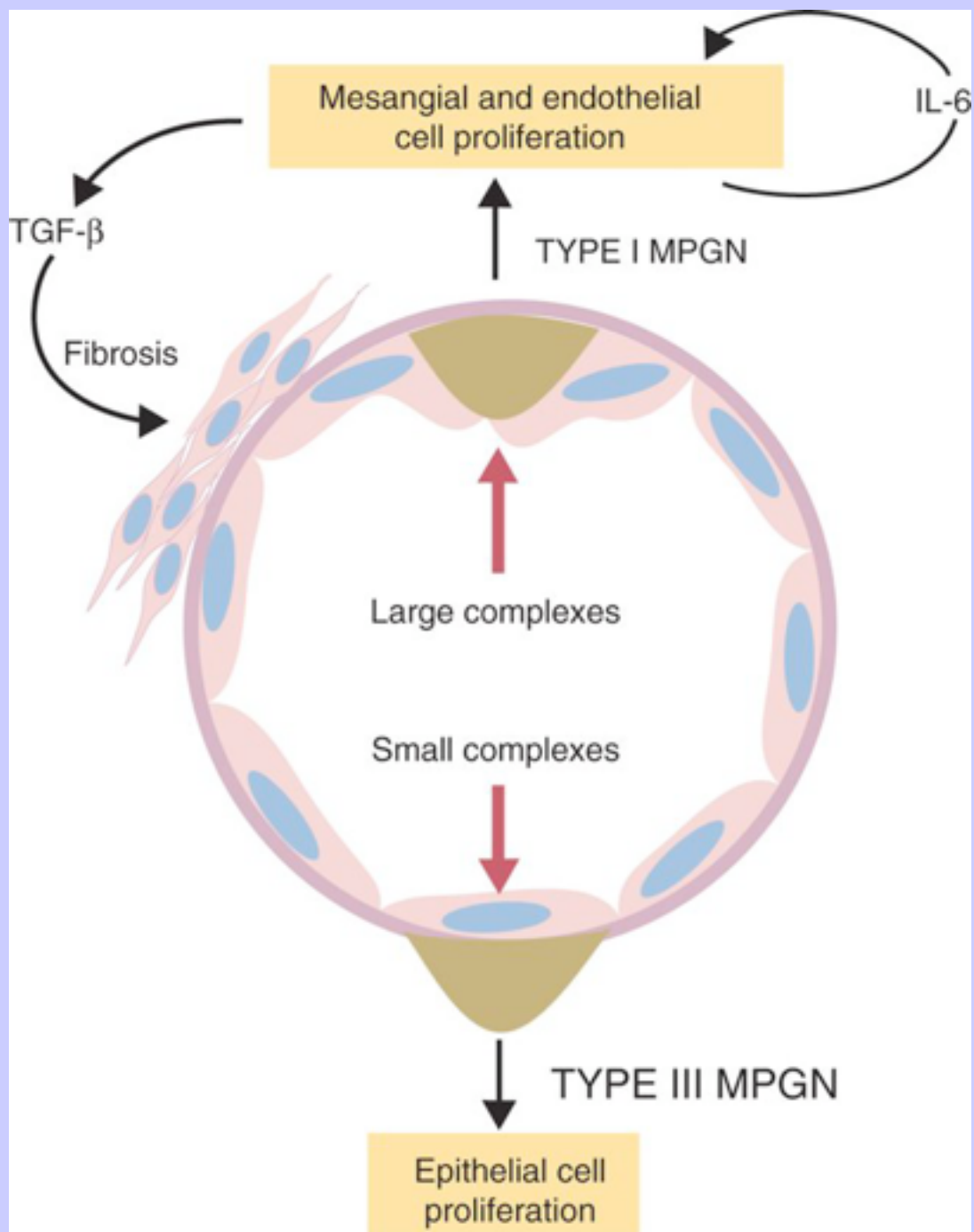
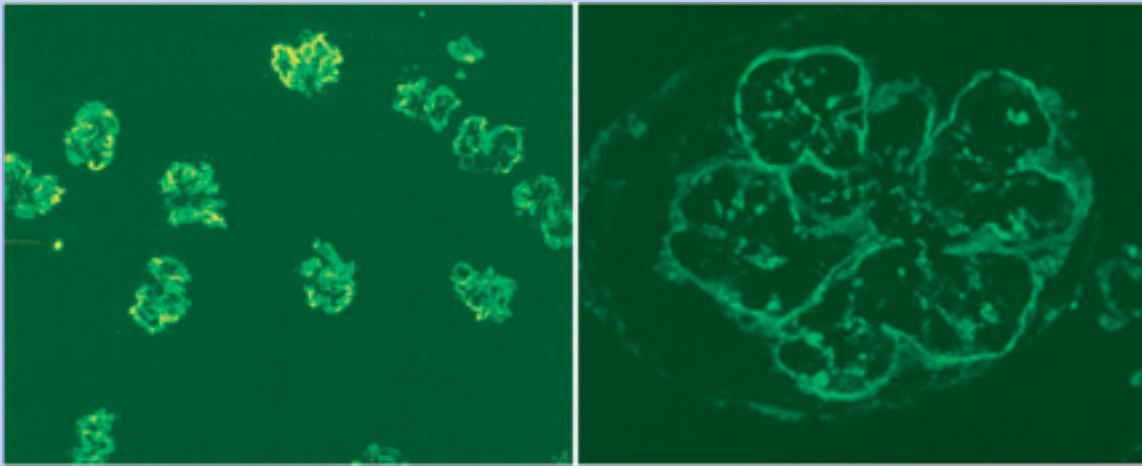


FIGURE 27-10 fluorescent micrographs of a section of glomerulus from a Finnish-Landrace lamb with immune complex-mediated glomerulonephritis. The labeled antisheep globulin reveals the presence of “lumpy-bumpy” deposits characteristic of type I membranoproliferative glomerulonephritis in many glomeruli. (From Angus KW, Gardiner AC, Morgan KT, et al: *J Comp Pathol* 84:319-330, 1974.)



of immune complexes is impaired and they accumulate in glomeruli. Many cases of type I MPGN develop in the absence of an obvious predisposing cause.

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Table 27-1 Infectious Diseases with a Significant Type III Hypersensitivity Component

Organism or Disease	Major Lesion
<i>Erysipelothrix rhusiopathiae</i>	Arthritis
<i>Mycobacterium johnei</i>	Enteritis
<i>Streptococcus equi</i>	Purpura
<i>Staphylococcus aureus</i>	Dermatitis
<i>Borrelia burgdorferi</i>	Glomerulonephritis
Ehrlichiosis	Glomerulonephritis
Canine adenovirus 1	Uveitis, glomerulonephritis
Canine adenovirus 2	Glomerulonephritis
Feline leukemia	Glomerulonephritis
Feline infectious peritonitis	Peritonitis, glomerulonephritis
Aleutian disease	Glomerulonephritis, anemia, arteritis
Hog cholera	Glomerulonephritis
African swine fever	Glomerulonephritis
Bovine virus diarrhea	Glomerulonephritis
Equine viral arteritis	Arteritis
Equine infectious anemia	Anemia, glomerulonephritis
Visceral leishmaniasis	Glomerulonephritis
<i>Dirofilaria immitis</i>	Glomerulonephritis

The presence of immune complex lesions within glomeruli stimulates cells such as neutrophils, mesangial cells, macrophages, and platelets to release thromboxanes, nitric oxide, and platelet-activating factor. These increase basement membrane permeability to macromolecules; as a result, plasma proteins, especially albumin, are lost in the urine. This loss, if severe, may exceed the ability of the body to replace the protein. As a result, albumin levels drop, the plasma colloid osmotic pressure falls, fluid passes from blood into tissue spaces, and the animal may become edematous and ascitic. The loss of fluid into tissues results in a reduction of blood volume, a compensatory increase in secretion of antidiuretic hormone, increased sodium retention, and accentuation of the edema. The decreased blood volume also results in a drop in renal blood flow, reduction in glomerular filtration, retention of urea and creatinine, azotemia, and hypercholesterolemia. Although all these may occur as a result of immune complex deposition in glomeruli, the development of this nephrotic syndrome is not inevitable. In fact, the clinical course of these conditions is extremely unpredictable, with some animals showing a progressive deterioration in renal function and others showing spontaneous remissions. Many animals may be clinically normal in spite of the presence of immune complexes in their glomeruli, and immune complexes are commonly observed in old, apparently healthy dogs, horses, and sheep. The most common initial signs are anorexia, weight loss, and vomiting. Polyuria and polydipsia occur when about two thirds of glomeruli are destroyed. Azotemia occurs when 75% are destroyed. Development of nephrotic syndrome (proteinuria, hypoproteinemia, edema, or ascites) only occurs in

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about 15% of affected dogs but in up to 75% of affected cats. Some dogs become hypertensive. Thromboembolic disease may also develop. Because of the unpredictable occurrence of spontaneous remissions, it is difficult to judge the effects of treatment. It has been usual to treat affected animals with corticosteroids and immunosuppressive drugs, but the rationale and effectiveness of this treatment are open to question except when the glomerulonephritis is associated with concurrent autoimmune disease such as systemic lupus erythematosus. Recently encouraging responses have been obtained with angiotensin-converting enzyme inhibitors (captopril) and experimental thromboxane synthase inhibitors. Protein restriction may help reduce the clinical signs of renal failure. If the glomerulopathy is secondary then clearly the underlying cause should be treated. The glomerular lesion is not inflammatory, and although the lesion in primary immune complex glomerulonephritis contains immunoglobulins, there is no evidence to suggest that it is caused by hyperactivity of the immune system. Steroid treatment of rabbits with experimental immune complex disease has been shown to exacerbate the condition.

27.5.1 IgA Nephropathy

By far the most important cause of renal failure in humans is IgA nephropathy. In this form of type I MPGN, patients have elevated serum IgA and IgA-containing immune complexes that are deposited in the mesangial region. The resulting cellular proliferation and glomerulonephritis can frequently lead to renal failure. The cause of IgA nephropathy is unknown. IgA deposits can be found in the glomeruli of up to 35% of some human populations and up to 47% of dogs. In these dogs, the IgA is deposited in the mesangial and paramesangial areas and is associated with mesangial proliferation. Dogs with enteritis or liver diseases show the highest incidence of glomerular IgA deposition. A slightly different condition has also been described in dogs aged 4 to 7 years. The animals developed a type III MPGN with mild hematuria, proteinuria, and hypertension. IgA-containing immune complexes formed in both the subepithelial and subendothelial locations. IgA nephropathy has also been described in pigtailed macaques (*Macaca nemestrina*).

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27.5.2 Swine Glomerulopathy

Spontaneous type I MPGN is observed in pigs. It is especially common in Japan, where it appears to be due to deposition of immune complexes containing IgG (and IgA) antibodies against *Actinobacillus pleuropneumoniae*. In other cases, it may be secondary to chronic virus infections such as hog cholera or African swine fever. Occasionally, however, a proliferative glomerulonephritis develops spontaneously. In most cases epithelial crescent formation suggests that the proliferating cells are epithelial in origin. However, occasional mesangioproliferative lesions are observed as well. There is usually strong staining for C3 and weaker staining for IgM using immunofluorescence assays. Pigs rarely have IgG or IgA deposits. Affected pigs are relatively young (less than 1 year). There is a high prevalence of gastric ulcers in affected animals, but whether this is related is unclear. An inherited complement factor H deficiency in Yorkshire pigs results in the development of a lethal type II MPGN called porcine dense deposit disease (see [Chapter 5](#)).

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27.5.3 Porcine Dermatitis and Nephropathy Syndrome

Porcine dermatitis and nephropathy syndrome is mainly seen in nursery and growing animals between 2 and 7 months of age. The clinical signs include weight loss, skin lesions, and, most commonly, sudden death. Clinically affected pigs may have high mortality, although this is highly variable. Skin lesions are seen in most cases. They present as flat or slightly raised multiple, reddish areas affecting the skin over the hamstrings, perineum, and ventral abdomen. In surviving animals, these lesions resolve in 2 to 3 weeks. The kidneys are enlarged, are congested, and may show multiple red spots. The skin lesions are associated with a widespread vasculitis involving medium and small arteries in the dermis and subdermis. Infarction leads to epidermal

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necrosis. The kidney lesions consist of a glomerulonephritis that may be acute and necrotizing or may be proliferative. Vasculitis is also seen in vessels in the kidney, lymph nodes, spleen, and liver. Some pigs may have renal lesions or skin lesions alone. This syndrome appears to be an immune complex disease affecting vascular epithelium. Immunoglobulins (IgG and IgM) and complement are deposited in and around the necrotic vessels in the early stages of the disease. The cause of the syndrome is unknown, but both bacteria and viruses have been implicated. Thus *Pasteurella multocida*-specific antigen has been isolated from affected kidney tissue. On the other hand, the lesions may be secondary to infections by porcine reproductive and respiratory syndrome virus or by porcine circovirus 2 (PCV2). The syndrome is commonly associated with porcine postweaning multisystemic wasting syndrome, a disease that may also be caused by PCV2 infection (see [Chapter 35](#)). Pigs suffering from the combined syndromes have higher morbidity and mortality.

27.5.4 **Dirofilariasis**

Some dogs heavily infected with the heartworm *Dirofilaria immitis* develop glomerular lesions and proteinuria. The lesions include thickening of the glomerular basement membrane with minimal endothelial or mesangial proliferation. Since IgG1-containing deposits may be found on the epithelial side of the basement membrane (type III MPGN), it has been suggested that immune complexes formed by antibodies to heartworm antigens provoke these lesions. Other investigators dispute the immune complex nature of this condition and claim that the lesions develop in response to the physical presence of microfilariae in glomerular blood vessels. The fact that infected dogs may develop amyloidosis (see [Chapter 4](#)) suggests that they mount a significant immune response to the worms.

27.5.5 **Finnish-Landrace Glomerulopathy**

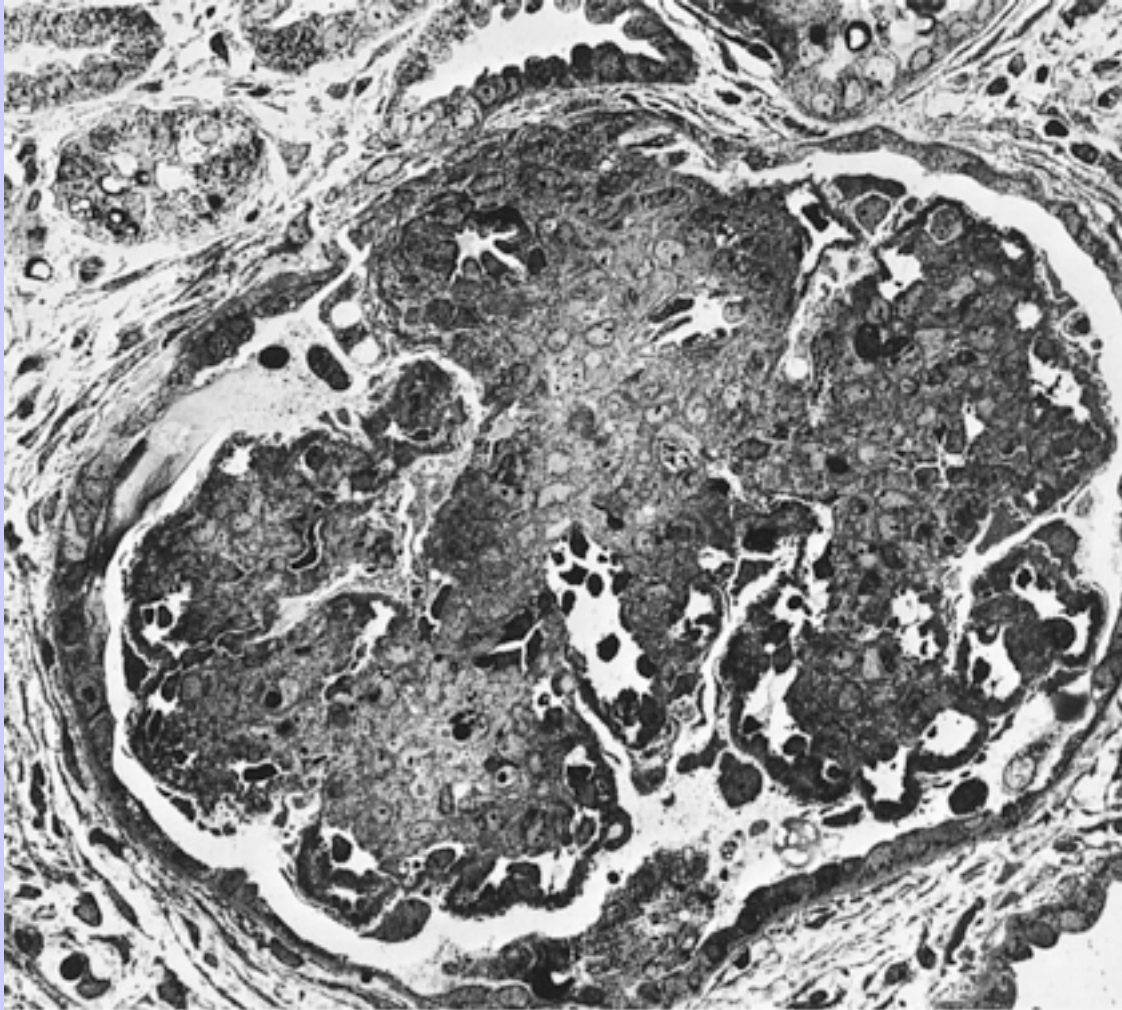
Some lambs of the Finnish-Landrace breed die when about 6 weeks of age as a result of renal failure due to a type I MPGN. The glomerular lesions are similar to those seen in chronic serum sickness, with mesangial cell proliferation and basement membrane thickening ([Figure 27-11](#)). In extreme cases epithelial cell proliferation may result in epithelial crescent formation. Neutrophils may be present in small numbers within glomeruli, and the rest of the kidney may exhibit diffuse interstitial lymphoid infiltration and necrotizing vasculitis. Deposits containing IgM, IgG, and C3 are found in the glomeruli and choroid plexus, and serum C3 levels are low. The lesions are therefore probably produced as a result of immune complex deposition within these organs, although the nature of the inducing antigen is unknown.

27.5.6 **Canine Glomerulopathy**

C3 deficiency inherited as an autosomal recessive condition has been described in Brittany Spaniels (see [Chapter 5](#)). Many of these dogs develop type I MPGN, which may result in renal failure. The lesions are typical with mesangial proliferation, thickening of the glomerular capillary wall, and deposition of electron-dense deposits in the mesangium and subendothelial space. The deposits contain both IgG and IgM. A familial glomerulopathy has been observed in Bernese

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FIGURE 27-11 A thin section of glomerulus from a Finnish-Landrace lamb with type I membranoproliferative glomerulonephritis. The primary lesion in this case is mesangial proliferation with some basement membrane thickening. (From Angus KW, Gardiner AC, Morgan KT, et al: *J Comp Pathol* 84:319-330, 1974.)



Mountain Dogs. It is associated with MPGN and interstitial nephritis.

27.6 OTHER IMMUNE COMPLEX-MEDIATED LESIONS

27.6.1 Purpura Hemorrhagica

Two to four weeks after an acute *Streptococcus equi* infection (or vaccination against *S. equi*), horses may develop urticaria, followed by severe subcutaneous edema, especially involving the limbs, and the development

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of hemorrhages in the mucosa and subcutaneous tissues. Affected horses are anorexic and depressed and have a high fever. Immune complexes containing *S. equi* antigens (M-protein or R-protein) may be found in the bloodstream of affected animals. These immune complexes cause an acute vasculitis, as well as a type I MPGN with resulting proteinuria and azoturia. Other triggers of purpura hemorrhagica in the horse include infections with *Corynebacterium pseudotuberculosis*, equine influenza virus, equine herpesvirus type 1, and *Rhodococcus equi*. In some cases it develops in the absence of any obvious infection. Horses usually recover if aggressively treated with systemic glucocorticosteroids.

Pigs may also suffer from sporadic cases of an immune complex-mediated thrombocytopenic purpura syndrome. The animals have thrombocytopenia, anemia, excessive bleeding, with membranoproliferative lesions in their glomeruli. The cause is unknown.

27.6.2 Dietary Hypersensitivity

If an antigenic milk replacer, such as soy protein, is fed to very young calves before the development of ruminal function, the foreign antigen may be absorbed and stimulate antibody formation and a type III hypersensitivity. As a result, the calves become unthrifty and lose weight. However, the precise pathogenesis of this condition is unclear. A small proportion of calves develop an IgE response and a type I hypersensitivity.

27.6.3 Polyarthritis

Immune complexes can be readily found in the blood and synovial fluid of animals with rheumatoid arthritis and in many with osteoarthritis. In rheumatoid arthritis they are believed to have a major role in the progression of disease. Their role in osteoarthritis is unclear, but they may be a secondary result of local trauma. Important examples of this type of arthritis are the nonerosive polyarthritides seen in foals and puppies and described in [Chapter 33](#).

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27.6.4 Drug Hypersensitivities

In the previous chapter, it was pointed out that if a drug attached itself to a cell such as an erythrocyte, the immune response against the drug could lead to elimination of the cell. A similar reaction may occur through type III hypersensitivity reactions if immune complexes bind to host cells. In this case, the cells are recognized as opsonized and are removed by phagocytosis. As might be predicted, if immune complexes bind to erythrocytes, anemia results; if they bind to platelets, thrombocytopenia and purpura result. Binding to granulocytes leads to a granulocytopenia and, consequently, recurrent infection. Severe skin reactions may follow deposition of antibody-drug complexes in the blood vessels of the dermis. However, in many cases it is difficult to distinguish between the toxic effects of a drug and type III hypersensitivity unless specific antibodies can be eluted from affected cells.

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28 CHAPTER 28 Type IV Hypersensitivity: Delayed Hypersensitivity

28.1 KEY POINTS

- Some antigens, when injected into the skin, induce a slowly developing inflammatory response called delayed, or type IV, hypersensitivity.
- Delayed hypersensitivity reactions are mediated by T cells and natural killer cells.
- A good example of a delayed hypersensitivity response is the reaction to intradermal tuberculin in cattle with tuberculosis. This tuberculin test provides a convenient diagnostic test for tuberculosis.
- A different form of type IV hypersensitivity occurs in allergic contact dermatitis. This is a slowly developing inflammatory response that occurs when reactive chemicals bind to skin cells and trigger a T cell response.

Certain antigens, when injected into the skin of sensitized animals, provoke slowly developing inflammation at the injection site. Since this “delayed” hypersensitivity reaction can only be transferred from sensitized to normal animals by lymphocytes, it must be cell mediated. Delayed hypersensitivity reactions are classified as type IV hypersensitivities and result from interactions involving the injected antigen, antigen-presenting cells, and T cells. An important example of a delayed hypersensitivity reaction is the tuberculin response. This is a skin reaction that develops in an animal infected with tuberculosis following an intradermal injection of tuberculin. Delayed hypersensitivity reactions can be considered to be a specialized form of inflammation directed against organisms that are resistant to elimination by conventional inflammatory processes.

28.2 THE TUBERCULIN REACTION

Tuberculin is the name given to extracts of mycobacteria used to skin-test animals in order to identify those suffering from tuberculosis. Several types of tuberculin have been employed for this purpose. The most important is purified protein derivative (PPD) tuberculin, prepared by growing organisms in synthetic medium, killing them with steam, and filtering. The PPD tuberculin is precipitated from this filtrate with trichloroacetic acid, washed, and resuspended in buffer ready for use. Thus PPD tuberculin is a crude antigen mixture. Its major antigenic component is probably the heat-shock protein HSP 65. Many of its proteins are shared among different mycobacterial species, thus ensuring that tests that use PPD tuberculin are relatively nonspecific.

When tuberculin is injected into the skin of a normal animal, there is no apparent response. On the other hand, if it is injected into an animal infected with mycobacteria, a delayed hypersensitivity response occurs. In these animals, a red, indurated (hard) swelling develops at the injection site. The inflammation begins between 12 and 24 hours, reaches its greatest intensity by 24 to 72 hours, and may persist for several weeks before fading gradually. In very severe reactions, tissue destruction and necrosis may occur at the injection site. Histological examination of the lesion shows that it is infiltrated with mononuclear cells (lymphocytes, macrophages), although neutrophils are present in the early hours of the reaction ([Figure 28-1](#)).

The tuberculin reaction is mediated by T cells. When an animal is infected with *Mycobacterium tuberculosis*, the organisms are readily phagocytosed by macrophages. Some of this mycobacterial antigen triggers a Th1 response and generates memory cells. These memory T cells will respond to injected mycobacterial antigens such as tuberculin. Since a positive tuberculin test can be elicited many years after exposure to an antigen, some of these memory T cells must be very long lived.

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When tuberculin is injected intradermally, it is taken up by Langerhans cells, which then migrate to the draining lymph node ([Figure 28-2](#)). Here they present antigen to memory T cells that respond by generating Th1 effector cells. The circulating Th1 cells recognize the antigen when they encounter it in the skin and accumulate around the antigen deposit. By 12 hours in cattle, the injection site is mainly infiltrated with γ/δ^+ , WC1⁺ T cells. (In humans and mice, α/β T cells tend to predominate, whereas in sheep and cattle, γ/δ T cells predominate.) There are no B cells in the lesion.

The γ/δ T cells help to recruit other Th1 lymphocytes and macrophages to the site. The Th1 cells secrete interferon- γ (IFN- γ), interleukin-2 (IL-2), and IL-

FIGURE 28-1 A histological section of a positive tuberculin reaction in bovine skin. Note the perivascular mononuclear cell infiltration as well as the lack of neutrophils or edema. (From Thomson RG: *General veterinary pathology*, Philadelphia, 1978, Saunders.)

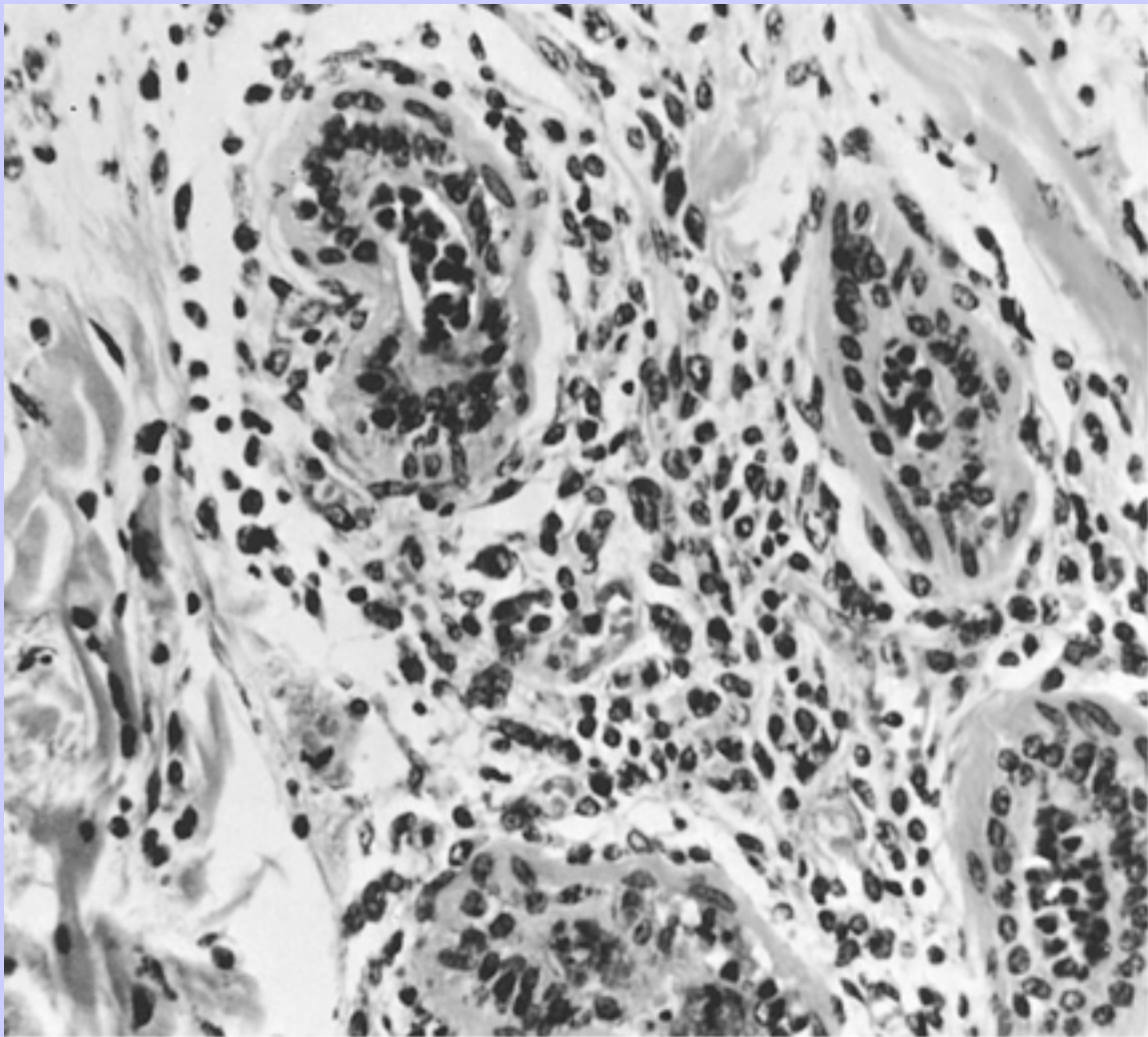
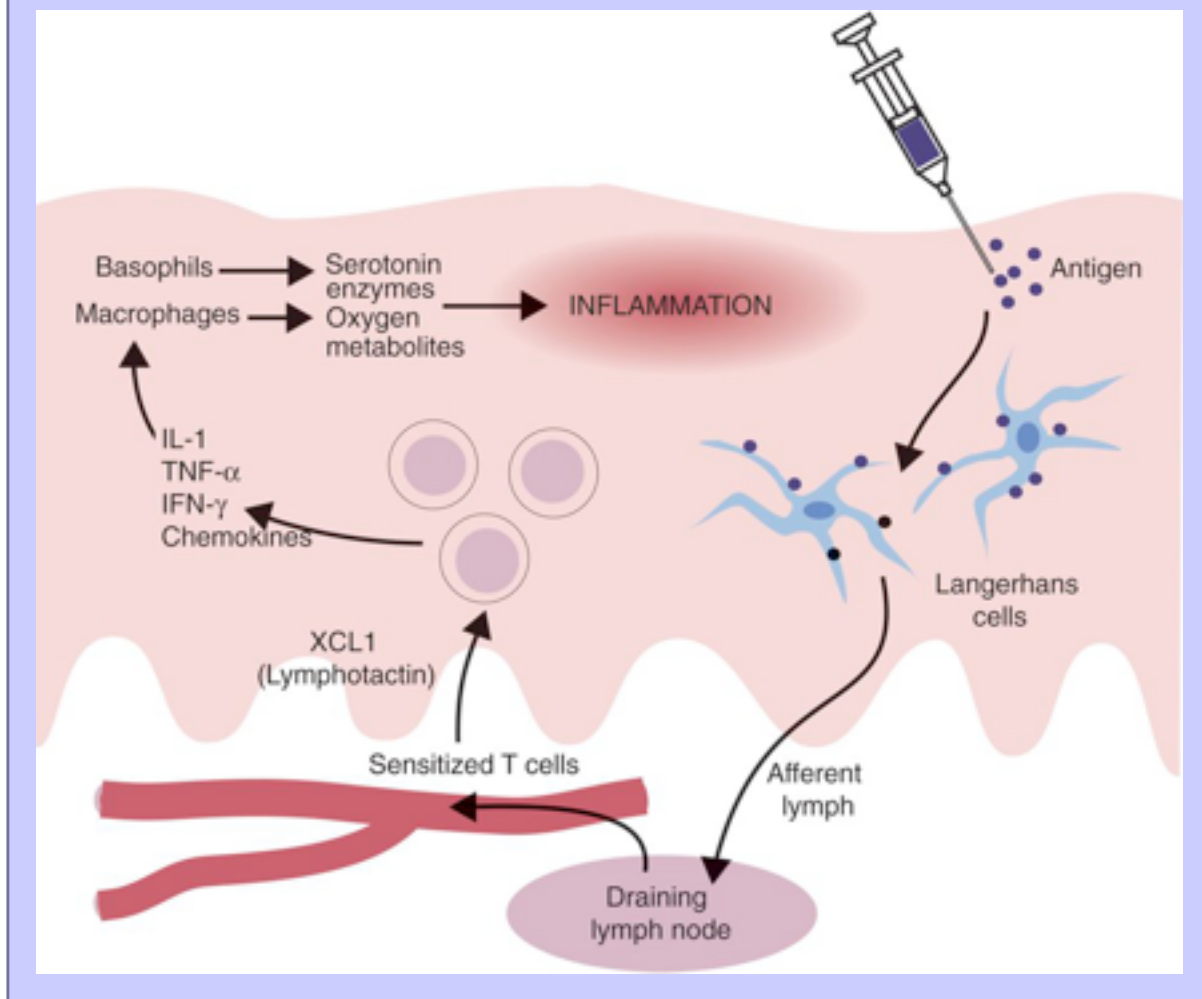
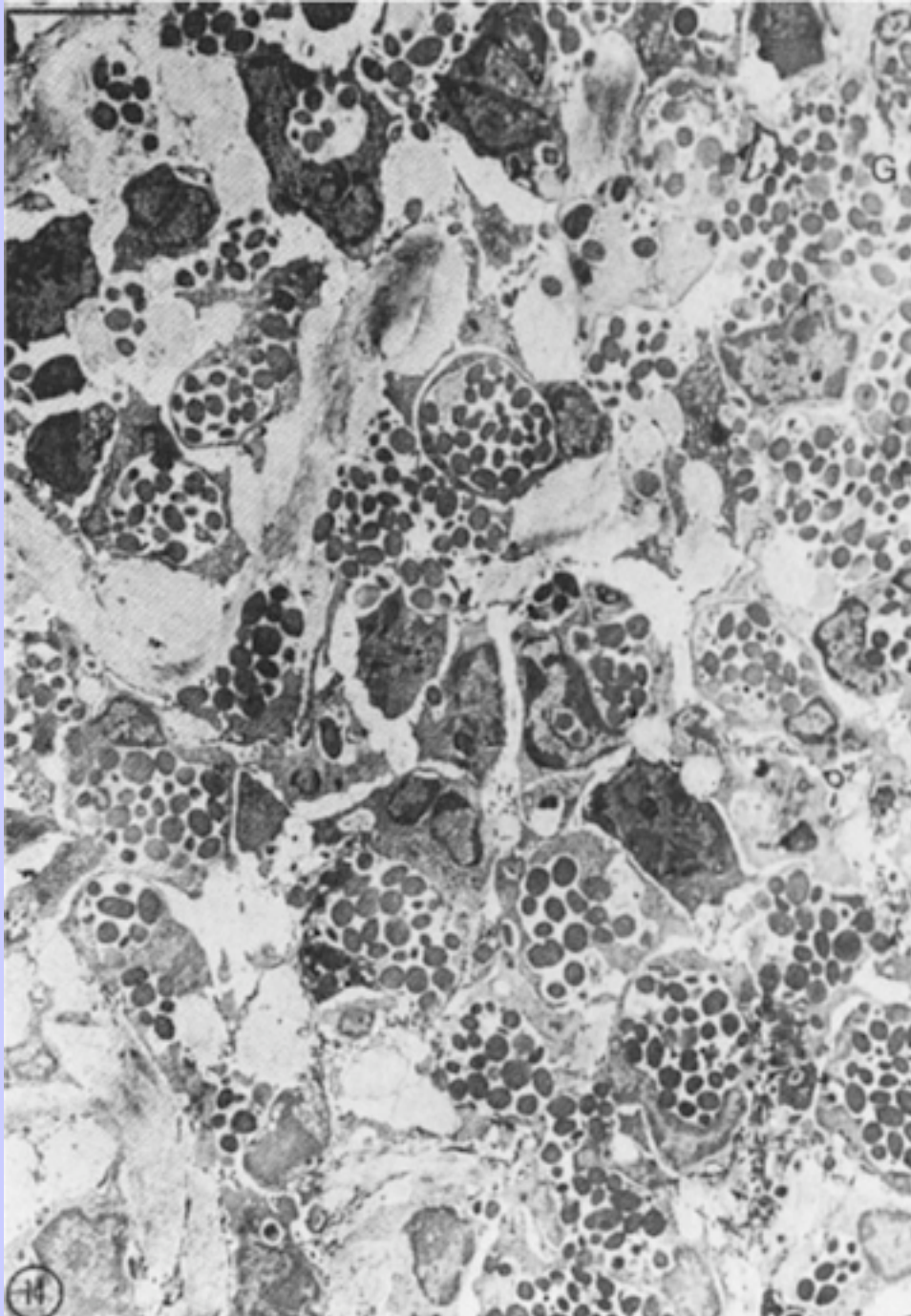


FIGURE 28-2 A schematic diagram depicting the mechanism of a delayed hypersensitivity reaction.



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FIGURE 28-3 A section of guinea pig skin 18 hours after attachment of a tick in an animal sensitized by prior infestation with tick larvae. The skin is infiltrated with large numbers of basophils. (From McLaren D, Worms MJ, Askenase PW: *J Pathol* 139:289, 1983.)



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16. The first two act on endothelial cells to increase expression of adherence molecules. IL-2 stimulates production of the chemokines CXCL8, CCL5, and XCL1, which attract and activate more T cells. IL-16 attracts CD4⁺ T cells. The macrophages also release serotonin and chemokines such as CXCL1 and CCL2, which attract basophils. Basophil-derived serotonin (in rodents) or histamine (in humans) causes yet more inflammation and enhances migration of mononuclear cells into the lesion. The T cell–derived chemokines CCL2 and CCL3 can induce mast cell degranulation, whereas some CD4⁺ T cells can activate mast cells directly through major histocompatibility complex (MHC) II–bound antigen.

T cell–derived chemokines cause inflammation and attract even more T cells. Most of these new T cells are not specifically sensitized for the inducing antigen. Only a very small proportion, perhaps 5%, of the lymphocytes seen in a delayed hypersensitivity reaction are specific for the antigen. The vast majority are attracted nonspecifically by XCL1. By 60 to 72 hours, the predominant lymphocytes are α/β^+ , CD4⁺, and CD8⁺. Macrophages accumulate in the lesion as a result of the production of CXCL8 and may be activated by IFN- γ . Some of the tissue damage in intense delayed hypersensitivity reactions may be due to the release of proteases and oxidants from these activated macrophages. The macrophages ingest and eventually destroy the injected antigen. This, plus the appearance of regulatory cells in the lesion, permits the tissues to return eventually to normal.

28.2.1 Cutaneous Basophil Hypersensitivity

Under some circumstances, basophils may be the predominant cells in a delayed hypersensitivity reaction ([Figure 28-3](#)). This type of reaction, called cutaneous basophil hypersensitivity (CBH), can be transferred between animals with antibody, with purified B cells, or even with T cells. CBH is therefore mediated by several different mechanisms. CBH occurs in chickens in response to intradermal Rous sarcoma virus, in rabbits in response to schistosomes, and in humans with allergic contact dermatitis and renal allograft rejection. CBH reactions may contribute to the development of flea allergy dermatitis in dogs.

28.3 TUBERCULIN REACTIONS IN CATTLE

Because a positive tuberculin reaction occurs only in animals that have, or have had, tuberculosis, skin testing may be used to identify animals affected by this disease. Indeed, the tuberculin test has provided the basis for all tuberculosis eradication schemes that involve the detection and subsequent elimination of infected animals.

Skin testing of cattle may be performed in several ways ([Table 28-1](#)). The simplest is the single intradermal (SID) test. In this test, 0.05 ml of PPD tuberculin derived from *M. tuberculosis* or *Mycobacterium bovis* is injected into one anal fold and the injection site is examined 72 to 96 hours later. A comparison is easily made between the injected and the uninjected folds, and a positive reaction consisting of a firm lump at the injection site is readily detected.

In the United States, two separate tests are performed. Two injections of tuberculin are made, one into the mucocutaneous junction of the vulva and the other into an anal fold. In other countries, tuberculin is normally injected into the skin on the side of the neck. The neck site is more sensitive than the anal folds, but restraint of the animal may be more difficult and good injection technique is critical.

The advantage of the SID test is its simplicity. Its main disadvantage is that because of cross-reactions it cannot distinguish between tuberculosis and infection by related mycobacteria such as *Mycobacterium avium*, *M. avium paratuberculosis*, or the *Nocardia* group of organisms. A second disadvantage is that some animals react positively

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to the test but on necropsy do not have detectable tuberculosis lesions. The reasons for this are unclear but may be a result of exposure to nonpathogenic mycobacteria such as *Mycobacterium phlei*.

Table 28-1 Tuberculin Tests Used in Cattle

Test	Usage	Advantages	Disadvantages
Single intradermal	Routine testing	Simple	Prone to false positives Poor sensitivity
Comparative	When avian TB or Johne's disease is prevalent	More specific than SID	More complex than SID
Short thermal	Use in postpartum animals and in infected animals	High efficiency	Time consuming Risk of anaphylaxis
Stormont	Use in postpartum animals and in advanced cases	Very sensitive and accurate	Three visits required May sensitize an animal

False-negative SID tests may occur in animals with advanced tuberculosis, in animals with very early infection, in animals that have calved within the preceding 4 to 6 weeks, in very old cows, and in animals tested during the preceding 1 to 10 weeks. The lack of reaction (anergy) seen in advanced cases of tuberculosis is also observed in clinical Johne's disease and appears to be due to the presence of a "blocking factor" in the serum of these animals. This factor may be an antibody that prevents T cells from reacting with antigen. There is also evidence for the involvement of regulatory cells in anergy. Because of these defects in the SID, several modifications of this test have been developed. The comparative test, for example, involves intradermal inoculation of both avian and bovine tuberculins. Each tuberculin is injected into the side of the neck at separate sites, and these sites are examined 72 hours later. In general, if the avian tuberculin site shows the greatest reaction, the animal is considered to be infected with *M. avium* or *M. avium paratuberculosis*. On the other hand, if the *M. bovis* site shows the greatest reaction, then it is believed that the animal is infected with *M. bovis* or *M. tuberculosis*. This test is useful when a high prevalence of avian tuberculosis or Johne's disease is anticipated. PPD from *M. bovis* is more specific in cattle than *M. tuberculosis*, giving less cross-reaction with *M. avium* as well as being more appropriate for use in cattle and is therefore preferred. In practice, recent evidence suggests that the comparative test has a sensitivity of 90% (10% false negatives) and a specificity of greater than 99% (less than 1% false positives); however, this depends on the criteria used to read the results.

Another modified tuberculin test is the short thermal test, in which a large volume of tuberculin solution is given subcutaneously and the animal examined for a rise in temperature between 4 and 8 hours later. (Presumably the tuberculin acts on T cells, which then provoke the release of IL-1 and other cytokines from macrophages.) The Stormont test relies on the increased sensitivity of a test site, which occurs after a single injection; it is performed by giving 2 doses of tuberculin at the same injection site 7 days apart. Both tests are relatively sensitive. As a result, they may be used in postpartum cows as well as for the testing of heavily infected animals. Repeated tuberculin testing results in a period of decreased reactivity and the induction of antibodies against *M. bovis* antigen HSP 70.

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28.4 TUBERCULIN REACTIONS IN OTHER ANIMALS

Tuberculin skin testing has never been a widely employed procedure in domestic animals other than cattle, so information on these animals is scanty. Nevertheless, it appears that the ability of different species to mount a classic tuberculin reaction varies greatly. In pig and cat, for example, the tuberculin test is unreliable, being positive for only a short period following infection. In pig and dog, the best test is an SID test given in the skin behind the ear, whereas in the cat the short thermal test is probably best. In sheep and goat, the antigen is usually given in the anal fold, but the results are usually unreliable in these species as well. Horses appear to be unusually sensitive to tuberculin, and the dose used must be reduced accordingly. Nevertheless, the results obtained do not always correlate well with the disease status of the animal. In birds, good reactions may be obtained by inoculating tuberculin into the wattle or wing web.

28.5 JOHNIN REACTIONS

Animals infected with *M. avium* var. *paratuberculosis*, the cause of Johne's disease, may develop a delayed hypersensitivity reaction following intradermal inoculation of an extract of this organism called johnin. Johnin can be used in a SID test but, like tuberculin, may give a negative result in animals with clinical disease. An intravenous johnin test is positive in these cases and may be a preferable alternative to the SID test. In this test the antigen is administered intravenously and the animal's temperature is noted 6 hours later. A rise in temperature of 1° C or neutrophilia is considered a positive result. These tests are probably of limited usefulness in individual animals but may help identify infected herds.

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28.6 OTHER SKIN TESTS

Positive delayed hypersensitivity skin reactions may be obtained in any infectious disease in which cell-mediated immunity has a significant role. Thus extracts of *Brucella abortus* have been used from time to time in attempts to diagnose brucellosis. These include brucellin, a filtrate of a 20-day broth culture, and brucellergen, a nucleoprotein extract. Because these preparations may stimulate production of antibodies to brucella, they cannot be employed in areas where eradication is monitored by serological tests. In glanders of horses, a culture filtrate of the organism *Pseudomonas mallei*, termed mallein, is used for skin testing. Mallein can be used in either a short thermal test or an ophthalmic test. An ophthalmic test, also occasionally employed in tuberculosis, is performed by dropping the antigen solution into an eye. Transient conjunctivitis develops if the test is positive. Another method of testing for glanders is the intrapalpebral test. In this test, mallein is injected into the skin of the lower eyelid, where a positive reaction results in swelling and ophthalmia.

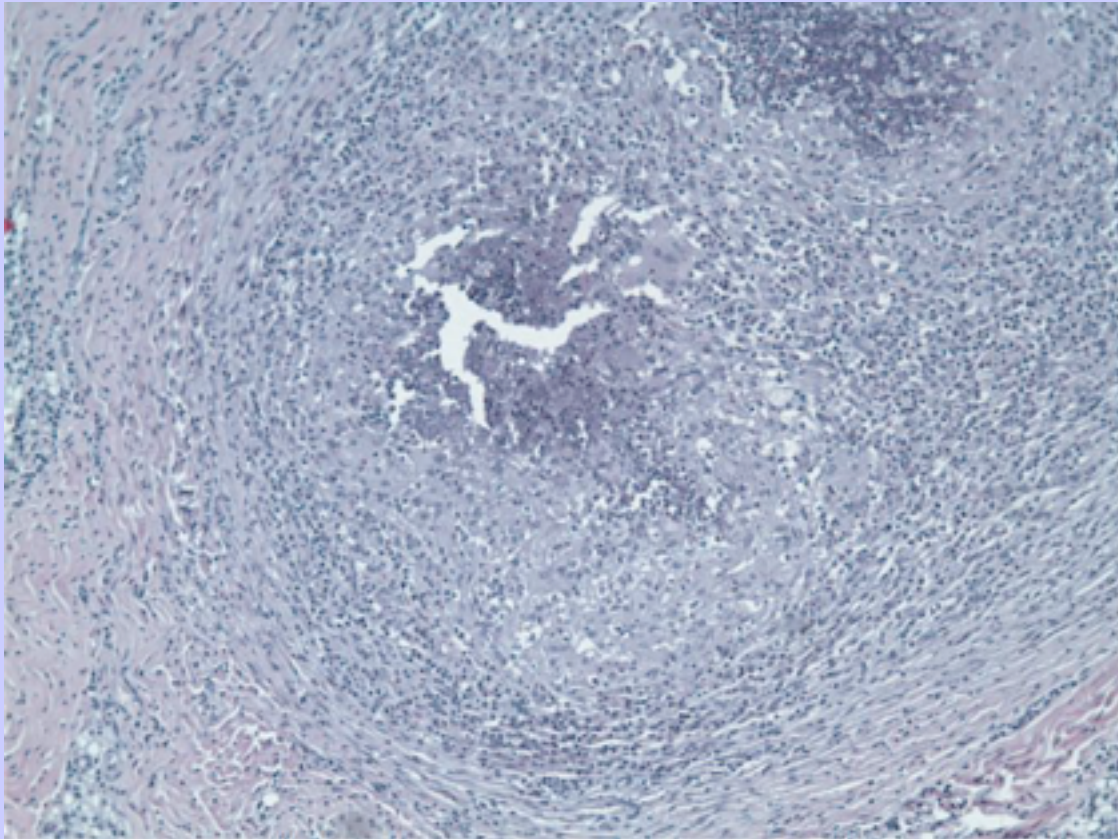
Intradermal skin testing with microbial extracts is also employed in the diagnosis of many fungal diseases; thus histoplasmin is used for histoplasmosis, coccidioidin in coccidioidomycosis, and so on. In these cases, the tests are not very specific, and the test procedure may effectively sensitize the tested animal, causing it to become serologically positive. This problem also arises when toxoplasmin is used in attempts to diagnose toxoplasmosis (see [Chapter 24](#)).

28.7 PATHOLOGICAL CONSEQUENCES OF TYPE IV HYPERSENSITIVITY

28.7.1 Tubercle Formation

Although the tuberculin reaction induced by intradermal inoculation is artificial in that antigen is administered by injection, a similar inflammatory response occurs if living tubercle bacilli lodge in tissues and sensitize an animal. However, *M. tuberculosis* is resistant to intracellular destruction until M1 macrophages are activated by Th1 cells (see [Chapter 16](#)), and dead organisms are very slowly removed because they contain large quantities of poorly metabolized waxes. As a result, the reaction to whole organisms is prolonged, and macrophages accumulate in very large numbers. Many of these macrophages ingest the bacteria but fail to prevent its growth and so die. Other macrophages fuse to form multinucleated giant cells. After 4 to 5 weeks of infection, microscopic granulomas enlarge and coalesce. The lesion that develops around invading tubercle bacilli therefore consists of a mass of necrotic debris containing both living and dead organisms surrounded by a layer of fibroblasts, lymphocytes, and macrophages, which in this location are called epithelioid cells (see [Chapter 4](#)). The entire lesion is called a tubercle ([Figure 28-4](#)). The mycobacteria are unable to multiply within the caseous tissue because of its low pH and lack of oxygen. Nevertheless, some bacteria may survive in a dormant state. If the host mounts an adequate immune response of the correct (Th1) type, this may be sufficient to control the infection. However, if immunity is insufficient or inappropriate (Th2), the organisms may escape from the tubercle and spread to local lymph nodes and nearby tissues. When the response is inadequate, the multiplying organisms continue to spread, and the resulting lung damage together with liquefaction of the caseous center of the tubercle leads to rapidly progressive disease. Granuloma formation is also a common result of persistent chronic inflammation. This inflammation may be of immunological origin, as in tuberculosis or brucellosis in some species, or it

FIGURE 28-4 A histological section from the lymph node of a cow infected with *Mycobacterium bovis* showing a small tubercle. The dark central mass is caseous material. It is surrounded by layers of macrophages and lymphocytes and walled off by fibroblasts. (Courtesy Dr. John Edwards.)



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may occur as a result of the presence in tissues of other chronic irritants. For example, granulomas may arise in response to the prolonged irritation caused by talc or asbestos particles.

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28.7.2

Allergic Contact Dermatitis

If reactive chemicals are painted onto the skin, they may bind to skin proteins and the resulting complexes are processed by Langerhans cells in the dermis ([Figure 28-5](#)). Depending on the antigen, the Langerhans cells may bind the antigen directly to MHC molecules on the cell surface or process the hapten internally into a complete antigen. The Langerhans cells then migrate to draining lymph nodes through afferent lymphatics and present the antigen to T cells. While presenting the antigen, the Langerhans cells secrete large amounts of IL-12 and IL-18, to which Th1 cells respond. These cells in turn produce large amounts of IFN- γ and promote the activities of cytotoxic T cells. Following exposure to an antigen in sensitized animals, macrophages and lymphocytes infiltrate the dermis by 24 hours. Eventually, the cytotoxic T cells destroy and remove the altered cells, resulting in the development of intraepithelial vesicles. This inflammatory reaction presents as an intensely pruritic skin

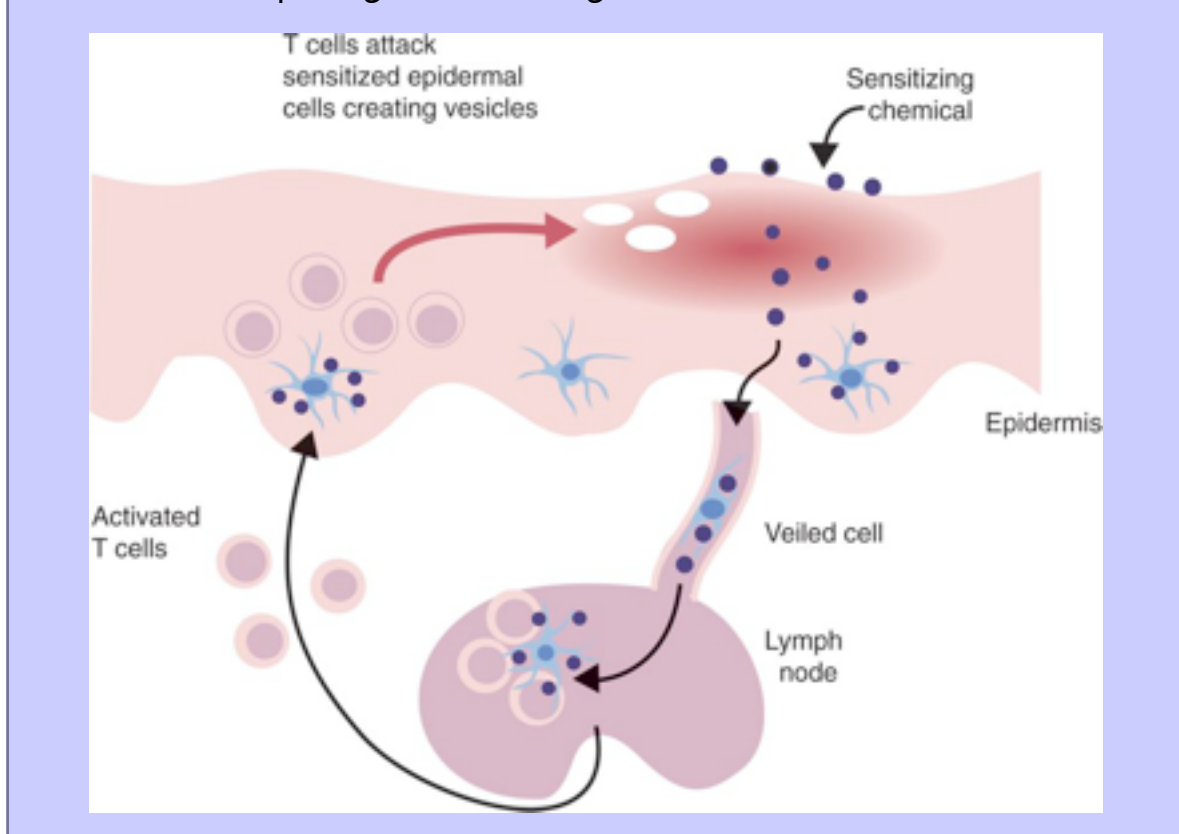
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disease called allergic contact dermatitis. In addition to α/β T cells, other cell types, such as γ/δ T cells, B-1 cells, and natural killer (NK) T cells, may be involved in the reaction.

Recent studies have demonstrated that contact dermatitis can be readily induced in mice that lack all types of lymphocytes except NK cells. In addition, contact dermatitis appears to be antigen-specific insofar as primed animals mount a much stronger response than unprimed animals. This appears to be a property of a subpopulation of NK cells. These NK cells can survive for at least 28 days in mice and so form a memory cell population. These results clearly are at variance with our previous ideas about the antigenic specificity of NK cells and their role in immunity. It is also of interest to note that contact dermatitis will not occur in skin that lacks functional nerve fibers. Clearly allergic dermatitis has a complex and poorly understood etiology.

The chemicals that induce allergic contact dermatitis are usually highly reactive molecules that combine chemically with skin proteins; they include formaldehyde, picric acid, aniline dyes, plant resins and oils, organophosphates, some topical medications such as neomycin, and salts of metals such as nickel and beryllium (Figure 28-6). Thus allergic contact dermatitis can occur on pathologists' fingers as a result of exposure to formaldehyde; on the ears of dogs treated with neomycin for otitis externa; on the foot pads, scrotum, and ventral abdomen of dogs on exposure to some carpet dyes and deodorizers; on parts of the body exposed to the oils (urushiol) of the poison ivy plant (*Rhus radicans*); and around the neck of animals as a result of exposure to dichlorvos (2,2-dichlorovinyl dimethylphosphate) in flea collars (Box 28-1). Severe lesions may develop on the teats of dairy cattle as a result of a contact dermatitis to a component of the rubber in a milking machine (*N*-isopropyl-*N*-phenyl diamine). Allergic contact dermatitis involving the muzzle of dogs has been reported to result from sen

FIGURE 28-5 The pathogenesis of allergic contact dermatitis.



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sensitivity to components of plastic food bowls. Some dogs, instead of developing the more usual type I hypersensitivity to pollen proteins, experience an allergic contact dermatitis as a result of a type IV hypersensitivity to pollen resins. It is unusual for allergic contact dermatitis to affect the haired areas of the skin unless the allergen is in a liquid. Thus allergic contact dermatitis to shampoo components may result in total-body involvement. The period required for sensitization ranges from 6 months to several years.

The lesions of allergic contact dermatitis vary in severity, ranging from a mild erythema to a severe erythematous vesiculation. However, because of the intense pruritus, self-trauma, excoriation, ulceration, and secondary staphylococcal pyoderma often mask the true nature of the lesion. If the exposure to the allergen persists, hyperkeratosis, acanthosis, and dermal fibrosis may eventually occur. Histologically, the lesion is marked by a mononuclear cell infiltration and vacuolation of skin cells under attack by cytotoxic T cells ([Table 28-2](#)).

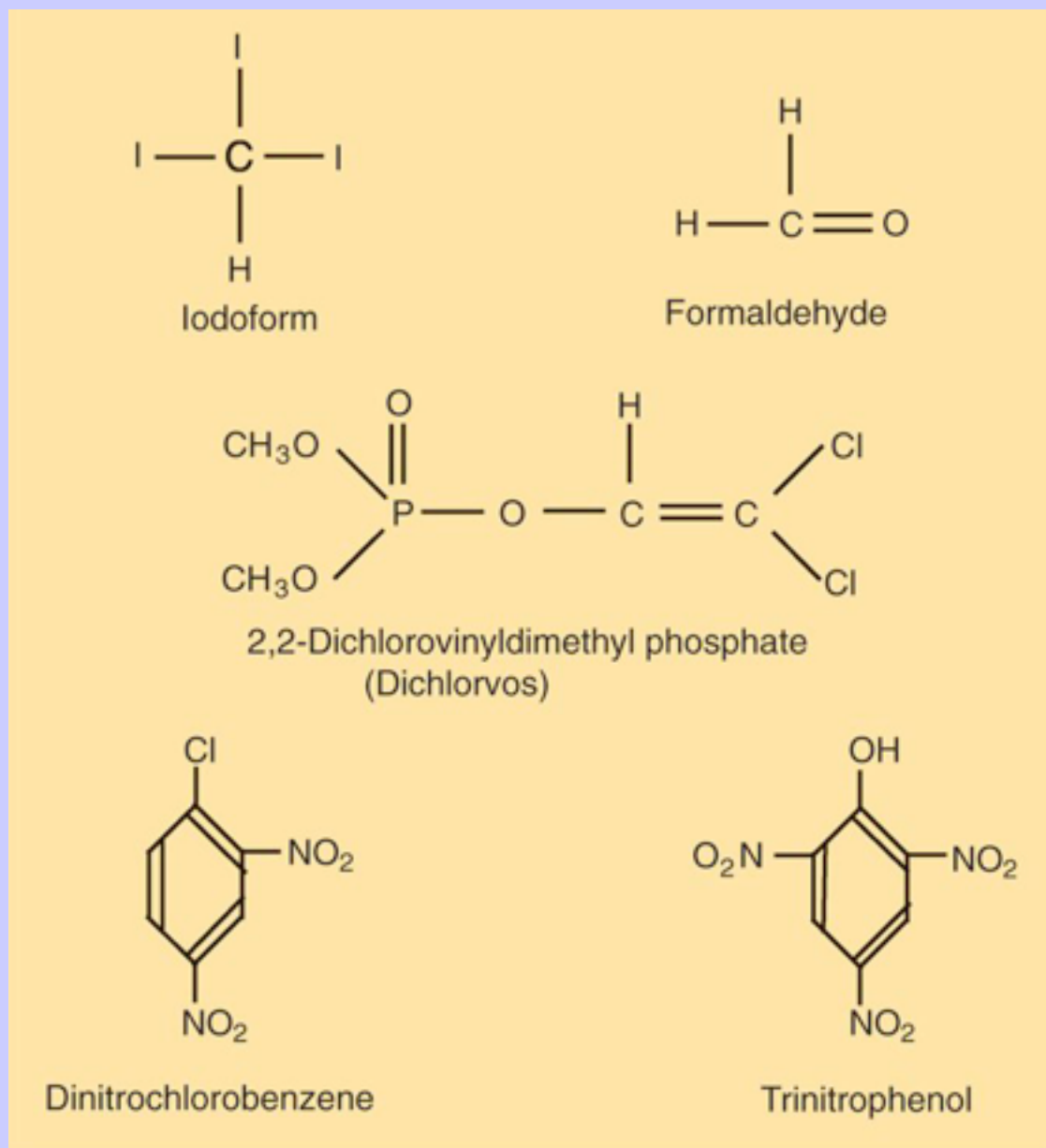
Allergic contact dermatitis is diagnosed by removal of the suspected antigen and by patch testing. In “closed” patch tests, suspected allergens are used to impregnate gauze swabs that are then attached to the shaved skin with tape. After 48 to 72 hours the dressing is removed and the areas in contact with the swabs examined. A positive reaction is indicated by local erythema and vesiculation. Closed patch tests may be impractical for some dogs and cats. An “open” patch test may therefore be employed. In this procedure, a solution of the suspected allergen is applied to shaved normal skin and the area examined daily for up to 5 days. Identification of the offending allergen and its avoidance by the animal are the optimal therapies for allergic contact dermatitis. Hyposensitization therapy is not effective. Steroids are used in acute cases, with antibiotics to control secondary infections.

28.7.3

Stevens-Johnson Syndrome

Three related mucocutaneous disorders—erythema multiforme, Stevens-Johnson syndrome, and toxic epi

FIGURE 28-6 Some of the simple chemicals that can cause allergic contact dermatitis.



dermal necrolysis—are well recognized in humans and have been diagnosed in dogs and cats. The three diseases are characterized by lesions of increasing severity. Erythema multiforme is characterized by patchy skin loss and low morbidity; Stevens-Johnson syndrome is more severe but involves less than 10% of the body surface; toxic epidermal necrolysis is much more serious with affected individuals losing more than 30% of their epidermis. Mortality is high. The three conditions however, overlap considerably. Stevens-Johnson syndrome and toxic epidermal necrolysis are believed to involve a T cell-mediated hypersensitivity to drugs. Erythema multiforme is not associated with drug administration. Affected animals develop vesicles, shed large areas of epidermis, and

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develop skin ulcers as a result of widespread apoptosis of their keratinocytes. The apoptosis is believed to result from drugs or their metabolites binding to the epidermal cells, upregulating CD95L expression as well as the production of soluble CD95L, and so triggering their destruction by cytotoxic T cells. Skin lesions are infiltrated mainly by CD8⁺ T cells and fewer CD4⁺ cells. Many different drugs may trigger these responses, but common inducers in dogs include trimethoprim-potentiated sulfonamides, beta-lactam antibiotics, penicillin, and cephalixin. Beginning about 14 days after drug exposure, the skin begins to blister and slough. Animals develop generalized illness including dyspnea, vomiting, fever, and weight loss. In dogs, sloughing of the epidermis occurs over the nasal planum, the footpads, and the oral, pharyngeal, nasal, conjunctival and preputial mucosa. Fluid loss leads to electrolyte imbalances while life-threatening secondary infections are common. Biopsies show extensive epidermal cell death.

Table 28-2 Comparison of the Major Forms of Allergic Dermatitis

	Atopic Dermatitis	Allergic Contact Dermatitis
Pathogenesis	Type I hypersensitivity	Type IV hypersensitivity
Clinical signs	Hyperemia, urticaria, pruritus	Hyperemia, vesiculation, alopecia, erythema
Distribution	Face, nose, eyes, feet, perineum	Hairless areas, usually ventral abdomen and feet
Major allergens	Foods and pollens, fleas, inhaled allergens	Reactive chemicals, dyes in contact with skin
Diagnosis	Intradermal testing, immediate response	Delayed response on patch testing
Pathology	Eosinophilic infiltration, edema	Mononuclear cell infiltration, vesiculation
Treatment	Steroids, antihistamines, hyposensitization	Steroids

28.7.3.1

Box 28-1 Sources of Contact Allergens in Animals

- Insecticides in flea collars
 - In sprays
 - In dips
- Wood preservatives
- Floor waxes
- Carpet dyes
- Some pollens
- Dermatological drugs (creams, ointments)
- Leather products
- Paints
- House plants

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Treatment involves immediate withdrawal of the offending drug followed by symptomatic treatment including fluid replacement. Corticosteroids should be avoided since they increase the animal's susceptibility to skin infections and worsen the prognosis. Antibiotics should only be administered if skin infections occur. Intravenous administration of high doses of human immunoglobulins have been used successfully to treat this disease in dogs. It is believed that these immunoglobulins block CD95/CD95-ligand interactions and so prevent keratinocyte apoptosis.

28.8 MEASUREMENT OF CELL-MEDIATED IMMUNITY

Although diagnostic immunology is based largely on the detection of serum antibodies, measurement of cell-mediated immune responsiveness in animals may be desirable under some circumstances. For example, in determining the effectiveness of a vaccine, one must take into account that serum antibody levels may not truly reflect the degree of immunity possessed by an animal. Animals without detectable antibodies may possess significant cell-mediated immunity. The term *cell-mediated immunity* encompasses a diverse set of mechanisms that employ T cells and macrophages for protection. Currently, both in vivo and in vitro techniques are used for this purpose.

28.8.1 In Vivo Techniques

The simplest in vivo test of cell-mediated immunity is an intradermal skin test such as the tuberculin test. The inflammation and swelling that occur in response to intradermally injected antigens may be considered cell mediated, provided that it has the characteristic time course and histological features of a type IV reaction. Intradermal skin tests are not always convenient, they are difficult to quantitate, and injection of an antigen may effectively sensitize an animal, thus preventing further testing.

It is sometimes useful to measure the ability of an animal to mount cell-mediated immune responses in general rather than to one specific antigen. One way to do this is to give the animal a small skin allograft and measure its survival time. A much simpler technique is to paint a small area of the animal's skin with a sensitizing chemical such as dinitrochlorobenzene. The intensity of the resulting allergic contact dermatitis provides a rough estimate of the animal's ability to mount a cell-mediated immune response.

If the T cell-stimulating lectin phytohemagglutinin is injected intradermally, it provokes a local tissue reaction with many features of a delayed hypersensitivity response. In pigs, for example, this reaction is characterized by infiltration with γ/δ^+ $CD4^-$ $CD8^-$ T cells. This is a very convenient and rapid method of assessing an animal's ability to mount a cell-mediated response without the need for first sensitizing the animal to an antigen. However, the response to phytohemagglutinin is nonspecific and its interpretation may be difficult.

28.8.2 In Vitro Techniques

In vitro tests are designed to measure the antigen-specific activation and proliferation of T cells. These also include their cytotoxic activities and their production of cytokines. All of these tests require that T cells be grown in cell culture; therefore few are useful in the field.

To measure T cell proliferation in response to an antigen, a suspension of purified peripheral blood lymphocytes from the animal to be tested is mixed with the antigen and cultured for 48 to 96 hours. Twelve hours before harvesting, thymidine labeled with the radioactive isotope tritium is added to the cultures. Normal, nondividing lymphocytes do not take up thymidine, but dividing cells do because they are actively synthesizing DNA. Thus,

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if the T cells are proliferating, they will take up the tritiated thymidine and their radioactivity will provide a measure of the amount of proliferation. The greater the response of the cells to an antigen, the greater will be their radioactivity. The ratio of the radioactivity in the stimulated cultures to the radioactivity in the controls is called the stimulation index. A related technique is to measure the proliferation of lymphocytes in response to mitogenic lectins (see [Chapter 11](#)). The intensity of the lymphocyte proliferative response, as measured by tritiated thymidine uptake, provides an estimate of the reactivity of an animal's lymphocytes.

Radioactive tritium may be replaced in proliferation assays by a simple colorimetric enzyme assay. Methylthiazoldiphenyltetrazolium bromide (MTT) is a pale yellow compound that serves as a substrate for active mitochondrial enzymes. The enzymes change the MTT color to dark blue. The intensity of this color change is a measure of the number of living cells in a culture. Thus in proliferation assays, the number of living cells increases, and this can be measured colorimetrically. The test is sufficiently sensitive to quantify the increase in T cell numbers triggered by antigen or mitogens.

To measure T cell-mediated cytotoxicity it is necessary to have a simple method of measuring cell death. This is usually based on the fact that living cells take up and retain chromium ions but if the cell dies the chromium is released into the extracellular fluid. Radioactive sodium chromate (^{51}Cr) may be used in this way to label target cells. Lymphocytes from an immune animal are mixed in an appropriate ratio with ^{51}Cr -labeled target cells. The mixture is then incubated for 4 to 24 hours at 37°C . At the end of this time, the cell suspension is centrifuged and the presence of ^{51}Cr in the supernatant measured. The amount of chromium released is related directly to the number of target cells killed. The amount of chromium released in the absence of cytotoxic cells must also be measured and subtracted from that released in the presence of cytotoxic cells in order to get a true reading.

A third in vitro assay is the measurement of cytokine release by T cells. One such technique involves assaying the release of IFN- γ by peripheral blood lymphocytes on exposure to tuberculin or to purified mycobacterial proteins. This technique has been developed as an alternative to the tuberculin test for the diagnosis of tuberculosis in cattle and deer. It involves adding tuberculin PPD to heparinized blood and incubating the mixture for 24 to 48 hours at 37°C . The plasma is then removed and assayed for any interferon produced, either by means of a simple bioassay or preferably by use of a sandwich enzyme-linked immunosorbent assay (ELISA) employing monoclonal antibodies. Three “antigens” are used: no antigen (negative control), *M. bovis* PPD, and *M. avium* PPD. The *M. avium* PPD is used to detect false-positive cross-reactions. Purified, recombinant mycobacterial proteins can reduce the incidence of false-positives even further. This technique has advantages over conventional tuberculin tests in that it does not compromise the immune status of the animal under test by injection of antigen. In addition, the animal does not have to be held for several days for the test to be read. It is also much simpler than other in vitro tests for cell-mediated immunity. The assay is at least as sensitive as the SID test and, if purified recombinant mycobacterial proteins are employed, is highly specific. (Its sensitivity is about 85%, and its specificity is as high as 90% to 99%.) Positive results are obtained earlier than by skin testing. However, it does appear to detect a slightly different population of animals than the skin test. It has also been successfully used to diagnose Johne's disease in sheep.

28.8.2.1

Enzyme-Linked Immunospot Assay

It is possible to use a variation of a sandwich ELISA (see [Chapter 38](#)) in order to determine the frequency of cytokine-secreting cells ([Figure 28-7](#)). In this assay a capture-antibody directed against the cytokine of interest is coated on the bottom of plastic tissue culture wells. The cells to be tested are cultured on this surface and exposed to the antigen of interest. Any cytokine secreted by these cells will bind to nearby capture-antibodies. Once the culture period is completed, the presence of this bound cytokine is detected by a conventional

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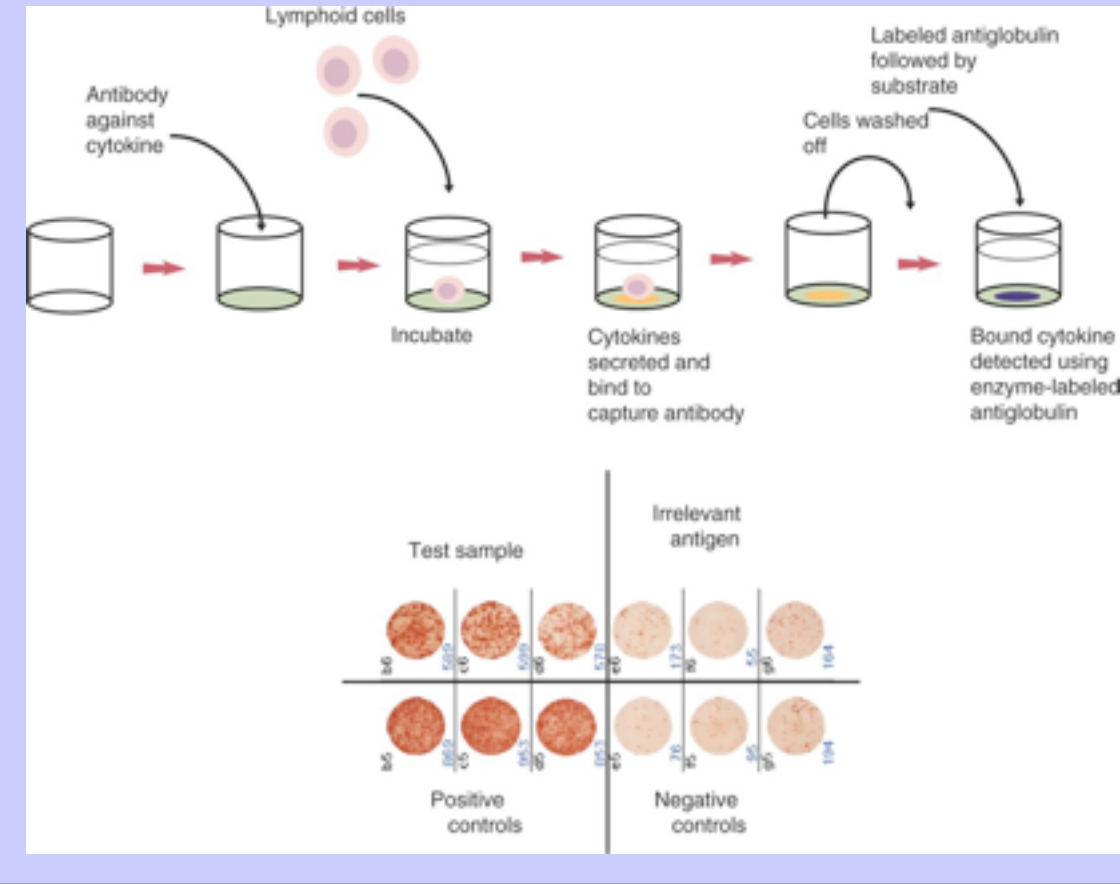
sandwich ELISA. This results in the development of a pattern of colored spots that correspond to the location of the cytokine-secreting cells. These spots can be counted and the frequency of specific cytokine-producing cells determined. This assay can also be used to quantitate cytotoxic cells by detecting granzyme or perforin production.

Although all of the assays described above can be used to measure at least some aspects of cell-mediated immunity, none provides a complete picture. The investigator may of course be simply interested in the response to a single antigen or organism. In these cases either a skin test or an in vitro assay may be appropriate. This is best exemplified by the tests available for the diagnosis of tuberculosis. In vitro tests are also useful if the time course of a cell-mediated immune response is to be examined. Repeated testing can be performed simply by obtaining more lymphocytes. If, on the other hand, an investigator wishes to obtain an overview of an animal's abilities in this area, then one of the nonspecific in vivo assays may be

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FIGURE 28-7 The principles of the enzyme-linked immunospot assay. The photograph shows the interferon- γ response of bovine peripheral blood mononuclear cells exposed to a defined *Anaplasma marginale* antigen. (Courtesy Dr. W. Mwangi.)



more appropriate. These can be useful, for example, in assessing immune function in young animals thought to be immunodeficient. However, it is important to point out that in these animals a complete hematological examination should be performed before more complex assays are considered. It is also prudent to measure

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the important lymphocyte subpopulations by flow cytometry. An animal that has no T cells is unlikely to mount any sort of cell-mediated response.

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29 CHAPTER 29 Organ Graft Rejection

29.1 KEY POINTS

- Organ grafts between two unrelated individuals of the same species are called allografts.
- Allografts are rejected by the recipient as a result of immune responses directed against donor blood group antigens and histocompatibility antigens.
- The response to donor histocompatibility antigens causes acute rejection and is primarily mediated by cytotoxic T cells attacking graft vascular endothelium.
- Chronic rejection and rejection directed against donor blood groups is mainly antibody mediated.
- Bone marrow stem cell allografts given to immunosuppressed recipients can attack the recipient to cause graft-versus-host disease.
- Some allografts such as those from the cornea are not rejected.
- The fetus can be considered to be an allograft but is not rejected as a result of multiple immunosuppressive mechanisms acting at the maternal-placental interface.

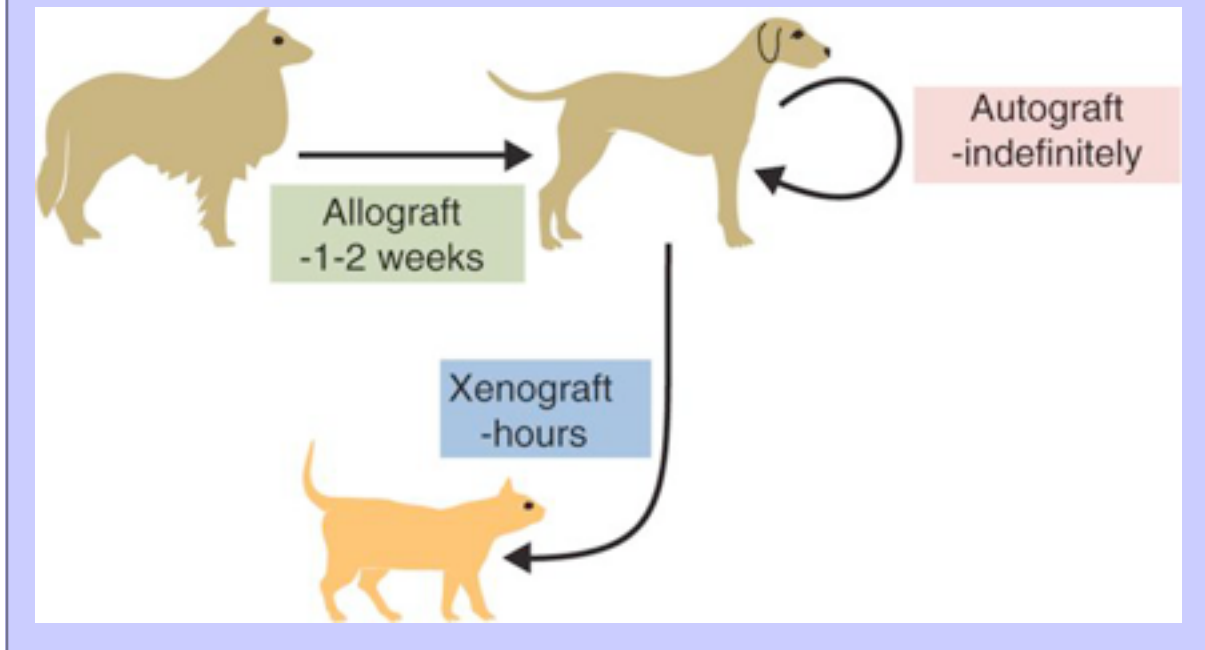
Although the immune response first attracted the attention of scientists because of the body's ability to fight infections, the observation that animals reject foreign organ grafts led to a much broader view of the immune system in that it indicated that the immune system had a surveillance function. The rejection of a foreign organ graft simply reflects the role of the immune system in identifying and destroying "abnormal" cells.

29.2 GRAFTING OF ORGANS

Advances in surgery have permitted the transfer of many tissues or organs between different parts of the body or between different individuals. When moved to a different part of an animal's own body, such transplants do not trigger an immune response. This type of graft within an individual is called an autograft ([Figure 29-1](#)). Good examples of autografting include the grafting of skin to cover a burn in plastic surgery and the use of a segment of vein to bypass blocked cardiac arteries. Since autografts do not express foreign antigens, they do not trigger an immune response.

Isografts are grafts transplanted between two genetically identical individuals. Thus a graft between identical (monozygotic) twins is an isograft. Similarly, grafts between two inbred mice of the same strain are isografts and present no immunological difficulties. Since the animals are identical, the immune system of

FIGURE 29-1 The different survival times of autografts, allografts, and xenografts.



the recipient cannot differentiate between the graft and normal body cells.

Allografts are transplanted between genetically different members of the same species. Most grafts performed on animals or humans for therapeutic reasons are of this type because tissues are obtained from a donor who is usually unrelated to the graft recipient. Because the major histocompatibility complex (MHC) and blood group molecules on the allograft are different from those of their host, allografts induce a strong immune response that causes graft rejection. This rejection process must be suppressed if the grafted organ is to survive.

Xenografts are organ grafts transplanted between animals of different species. Thus the transplant of a baboon heart into a human infant is a xenograft. Xenografted tissues differ from their host both biochemically and immunologically. As a result, they can provoke a rapid, intense rejection response that is very difficult to suppress.

Clinical grafting in domestic animals is a very recent procedure. However, renal allografting is now routine in dogs and cats, and bone marrow allografts promise to be very useful in some forms of tumor therapy. It is highly unlikely that cadaveric allografting will become important in veterinary medicine. Most current organ grafts are obtained from healthy donor animals. This raises significant ethical issues as to whether it is appropriate to subject a donor animal to major surgery in order to provide an organ for another animal. While the benefits of allografting to the recipient are obvious, it is unclear how the donor animal might benefit. Unlike human donors driven by altruism, an animal donor is given no choice in the matter. It is possible, however, to justify organ donation if an animal would thereby be saved from inevitable euthanasia and if the donor could be provided with a good home. For this reason, many animal transplantation centers require that the donor animal be adopted and cared for by the owner of the recipient animal.

29.3 ALLOGRAFT REJECTION

The identification and destruction of foreign molecules are central to the body's defense. Allografted organs represent a major source of these foreign molecules. They include not only antigens such as the foreign blood group glycoproteins and MHC molecules expressed on the grafted cells, but also any endogenous antigens presented on the MHC class I molecules of these same cells. The mechanisms of allograft rejection are basically the same irrespective of the organ grafted, and both antibodies and T cells participate in the rejection of allografts.

Renal allograft rejection is of major clinical importance in humans and has been widely studied in animals. It therefore serves as a good example of the allograft response. Rejection may occur at any time following transplantation. However, it is convenient to classify rejection as either acute or chronic since these have different underlying mechanisms. Acute rejection occurs within weeks or months of transplantation and is mediated by cytotoxic T cells. Histologically there is a mononuclear infiltrate of the kidney together with arterial wall necrosis. Acute rejection should be suspected when the recipient shows rapidly rising blood creatinine associated with an enlarged, painful kidney accompanied by signs of depression, anorexia, vomiting, proteinuria, hematuria, and ultrasonography showing an enlarged, hypoechoic kidney. In contrast, chronic rejection should be suspected if the creatinine and urea levels rise gradually and this is associated with proteinuria, microscopic hematuria and a small, hyperechoic kidney. It is associated with a slow loss of renal function and tends to be associated with interstitial fibrosis and proliferation of vascular endothelium. Renal biopsy is necessary to confirm rejection. In humans, where a great deal of experience with transplantation has been gained, four distinct clinical rejection syndromes are recognized. *Hyperacute rejection* occurs within 48 hours following grafting. Rejection occurring up to 7 days after grafting is called accelerated rejection. Rejection after 7 days is called *acute rejection*. *Chronic rejection* develops several months after grafting. It is unclear whether a similar classification is useful in animals.

29.3.1 Histocompatibility Antigens

When an organ is transplanted into a genetically dissimilar animal, the recipient will mount an immune response against many different antigens in and on the cells of the allograft. These are called histocompatibility antigens. Three types of histocompatibility antigens are of major importance in stimulating graft rejection. These are the MHC class I and class II molecules and the major blood group molecules. All are expressed on the surface of the graft cells, but their distribution varies. Thus MHC class I antigens are found on almost all nucleated cells. The major blood group antigens are found both on red cells and nucleated cells. MHC class II antigens, in contrast, have a restricted distribution that varies among mammals. For example, in rats and mice, MHC class II molecules are expressed only on the professional antigen-presenting cells: macrophages, dendritic cells, and B cells. In other species, such as humans and pigs, MHC class II molecules are also expressed on the endothelium of renal arteries and glomeruli, the sites where host cells first make contact with the graft. These MHC class II molecules are recognized as foreign and trigger the rejection process. It is interesting to note that, as a result of these differences, it is much easier to prolong renal allograft survival in laboratory rodents than in humans or pigs.

As would be expected, grafts that differ minimally from the recipient will generally survive longer than grafts that are highly incompatible. Thus when blood group A-O-compatible pigs are given renal allografts, median survival is about 12 days for completely unmatched grafts, 25 days for grafts compatible for MHC class I alone, 29 days for grafts compatible for MHC class II alone, and 80 days for grafts compatible for both class I and class II (Figure 29-2). When dogs are given MHC-unmatched renal allografts, the grafts survive for about 10 days. Completely matched allografts in dogs survive for about 40 days. A more impressive result is obtained with

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canine liver grafts, which survive for about 8 days in unmatched animals and for 200 to 300 days in DLA-matched recipients.

The failure of MHC and blood group-compatible grafts to survive indefinitely is a result of the cumulative effects of many other minor antigenic differences. For example, skin grafts from male donors placed on histocompatible females are usually rejected, although the reverse is not the case. This is because male cells carry an antigen, called the H-Y antigen, coded for by genes on the Y chromosome.

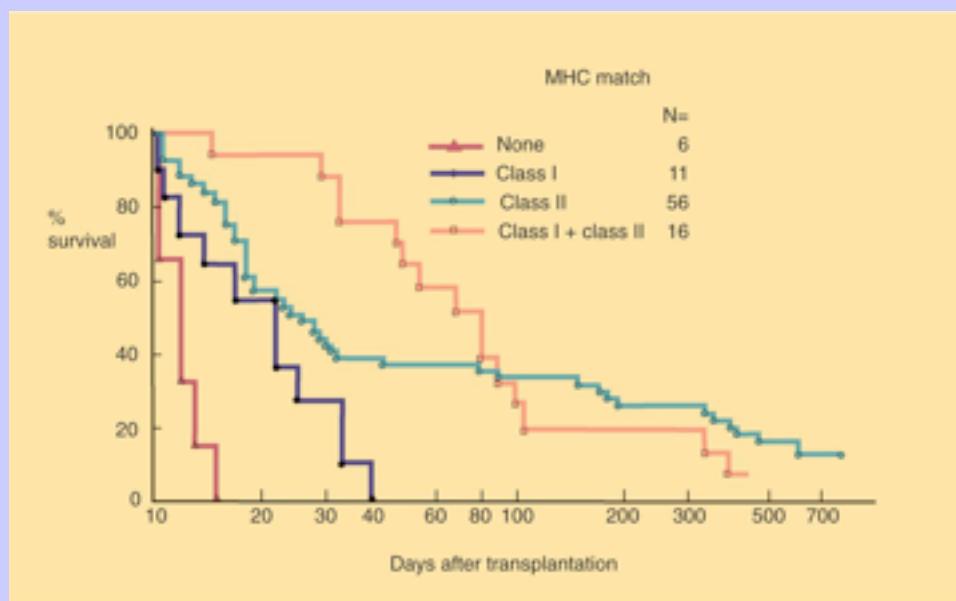
In practice it is usually not difficult to ensure that the donor and recipient have identical major blood group antigens. MHC compatibility is much harder to achieve because the extreme MHC polymorphism ensures that individuals differ widely in their MHC haplotype. In general, the more closely donor and recipient are related, the less will be their MHC difference. For this reason it is preferable that grafts be obtained from a recipient's parents or siblings. If this is not possible, then a donor must be selected at random and the inevitable rejection responses must be suppressed by drugs such as cyclosporine or tacrolimus (see [Chapter 36](#)).

29.4 RENAL ALLOGRAFTS

29.4.1 Pathology of Allograft Rejection

When kidneys are allografted, the blood supply to the transplanted kidney is established at the time of transplantation. Thus the graft and host cells come into

FIGURE 29-2 Survival time of organ allografts between swine leukocyte antigen-incompatible minipigs clearly depends on the degree of major histocompatibility complex compatibility between donor and host. (From Pescovitz MD, Sachs DHJ: *J Exp Med* 160:1493, 1994.)



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contact almost immediately. Damage to the graft through oxidant stress and surgical trauma causes the release of chemokines that attract inflammatory cells such as neutrophils and macrophages to the graft. In an unsensitized host, a primary immune response (first-set reaction) is mounted and renal allografts are rejected only after at least 10 days and possibly much longer. In sensitized animals, where the immune system is already primed, hyperacute rejection occurs and the graft is destroyed within days or even hours without ever becoming functional.

During an acute rejection process, the whole organ gradually becomes infiltrated with mononuclear cells, especially cytotoxic T cells, that cause progressive damage to the endothelial cells lining small intertubular blood vessels ([Figure 29-3](#)). T cell-mediated damage releases chemokines that attract more T cells into the graft. Tubular destruction, stoppage of blood flow, hemorrhage, and death of the grafted kidney follow thrombosis of these vessels. The blood vessels of second kidney grafts rapidly become blocked as a result of the action of antibodies and complement on the vascular endothelium. This leads to decreased urine production and stoppage of renal function. This “second-set” reaction is specific for any graft from the original donor or from a donor syngeneic with the first. It is not restricted to any particular site or to any specific organ since MHC and blood group molecules are present on most nucleated cells.

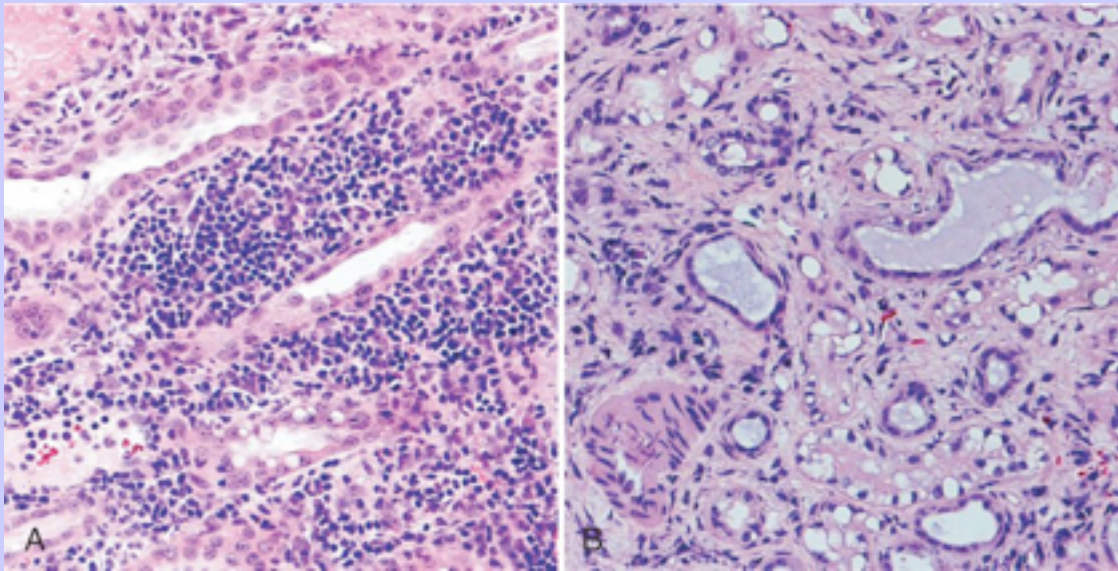
29.4.2 Mechanisms of Allograft Rejection

The allograft rejection process is directed against the dominant antigens on the cells of the graft. The MHC molecules tend to trigger a T cell-mediated rejection response whereas the blood group antigens tend to trigger antibody formation. The rejection process may be divided into two stages. First, the antigens of the graft encounter the host's antigen-sensitive cells and trigger a response. Second, cytotoxic T cells and antibodies from the host enter the graft and destroy graft cells ([Figure 29-4](#)).

Minor tissue damage during surgery probably permits alarmins such as high mobility group box protein-1 to activate toll-like receptors and trigger inflammation. Dendritic cells are activated and present graft antigens to recipient Th1 cells. As a result, the Th1 cells secrete interleukin-2 (IL-2) and interferon- γ (IFN- γ) and activate cytotoxic T cells and natural killer (NK) cells. The NK cells produce more IFN- γ and tumor necrosis factor- α (TNF- α), which activates effector cells especially macrophages and additional NK cells. The cytotoxic CD8⁺ T cells recognize and respond to the foreign proteins made by the graft cells. Their T cell antigen receptors (TCRs) recognize the foreign peptides bound to MHC class I molecules and trigger a cytotoxic T cell response. Allografted MHC class II molecules trigger an immune response in two ways. First, they are foreign proteins made by graft cells, so they themselves are processed as endogenous antigens. Second, intact graft MHC molecules may directly bind and trigger recipient TCRs. The recipient's cytotoxic T cells will therefore attack target cells bearing foreign class I MHC molecules on their surface.

Host Th1 cells secrete IL-2 and IFN- γ . These cytokines stimulate cytotoxic T cell activity and enhance the expression of MHC molecules on the cells of the graft. During allograft rejection, therefore, MHC expression is increased and the graft becomes an even more attractive target for cytotoxic T cells.

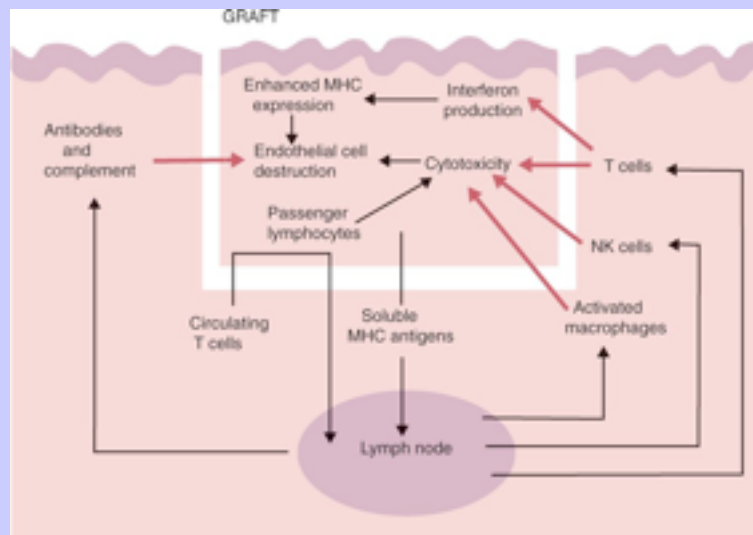
FIGURE 29-3 **A**, Section of a canine kidney that had been acutely rejected and as a consequence is densely infiltrated with lymphocytes. **B**, Section of a kidney that has undergone chronic allograft rejection. In this case the section shows interstitial fibrosis with tubular atrophy and a mild lymphocytic infiltration. (Courtesy Dr. A.E. Kyles.)



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FIGURE 29-4 Some of the mechanisms involved in the rejection of an allograft (see text for details).

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Blood group antigens mainly stimulate Th2 cells and antibody formation. Released by the graft cells, they are processed as exogenous antigens and so trigger B cell responses. Since animals may have preexisting “natural” antibodies to some of these blood group antigens, they may trigger hyperacute rejection.

29.4.2.1

Sensitization of the Recipient

Allograft recipients may be sensitized by a direct pathway when recipient T cells pass into the blood vessels of the graft, encounter foreign MHC class I and class II molecules on endothelial cells, and respond to them. Alternatively, soluble antigens may leak from the grafted organ and sensitize the recipient indirectly. For example, graft cells may release soluble MHC peptides that are recognized by the recipient's immune system. In humans, the direct pathway is responsible for the vigorous immune response that occurs in acute rejection whereas this indirect pathway is more important in chronic rejection.

In laboratory rodents, MHC class II molecules are expressed on professional antigen-presenting cells. In these species, therefore, the intensity of graft rejection is related to the number of donor B cells, macrophages, and dendritic cells transplanted within the graft. Previous removal of these cells by careful flushing of the graft before surgery or by pretreatment of the donor with cytotoxic drugs greatly reduces the intensity of the rejection process. In other mammals where MHC class II molecules are also expressed on vascular endothelial cells, these “passenger” cells are of less significance.

The cells that recognize the MHC molecules on graft cells move to the draining lymph node and activate other T cells. The paracortical regions of lymph nodes draining a graft therefore contain increased numbers of dividing lymphocytes. The numbers of these cells are greatest about 6 days after grafting and decline rapidly once the graft has been rejected. In addition to these signs of an active T cell–mediated immune response, it is usual to observe germinal center formation in the cortex and plasma cell accumulation in the medulla, indicating that antibody formation is also occurring.

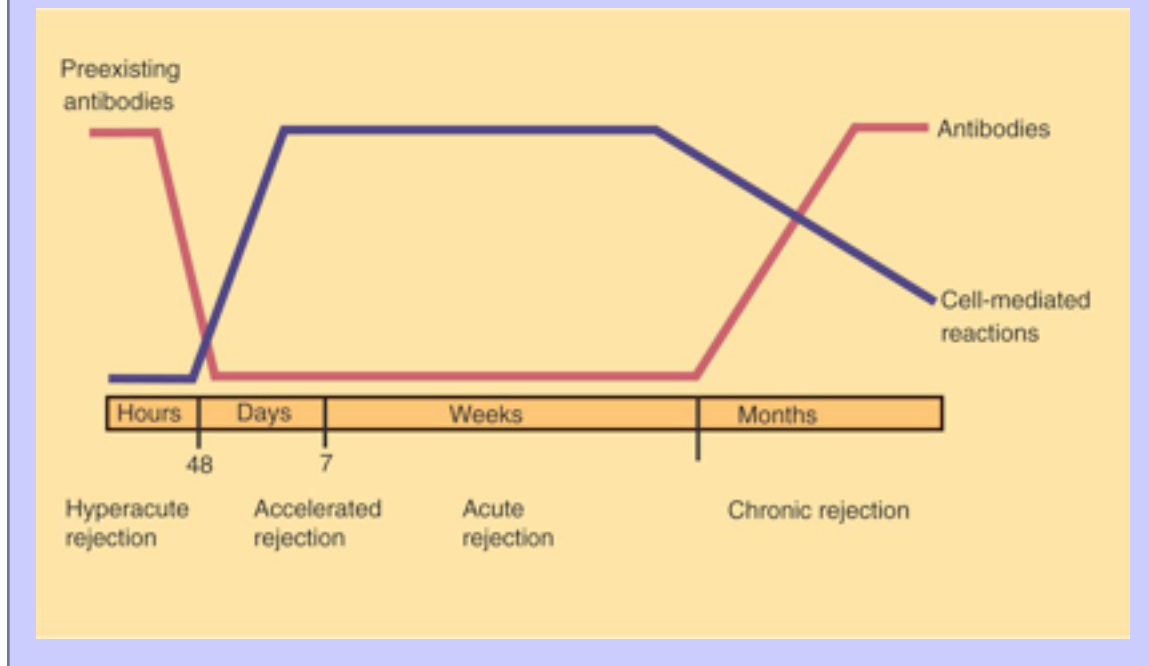
29.4.2.2

Destruction of the Graft

Once activated by exposure to antigen, CD8⁺ T cells reach the graft through the bloodstream. When these T cells enter the graft, they bind and destroy vascular endothelium and other accessible cells. As a result of this damage, hemorrhage, platelet aggregation, thrombosis, and stoppage of blood flow occur. The grafted tissue dies because of the failure of its blood supply. CD4⁺ T cells that enter the graft may release cytotoxic cytokines such as TNF- α . If renal allografts are biopsied and the lymphocytes within them examined, host CD8⁺ T cells predominate early in the allograft response whereas CD4⁺ T cells tend to dominate later in the response.

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FIGURE 29-5 Role of antibodies and cell-mediated immunity in different allograft rejection syndromes.



Although cytotoxic T cells are of major importance in acute allograft rejection, antibodies also play a significant role in hyperacute and chronic rejection ([Figure 29-5](#)). Antibody formation is especially important when the donor and recipient differ in their major blood groups. Antibodies also play a major role in the second-set reaction, where they may be directed against MHC class I molecules on the graft. These antibodies activate the classical complement pathway leading to target cell lysis. They also act through neutrophils and other cells with Fc receptors to mediate antibody-dependent cytotoxic cell activity.

29.4.3 Prevention of Allograft Rejection

In preventing allograft rejection, the transplantation surgeon seeks to cause sufficient immunosuppression while at the same time not making the recipient more susceptible than necessary to infectious agents. Dogs mount very strong allograft responses, and kidney allografts are rejected in 6 to 14 days in untreated animals. Unrelated dogs with renal allografts show about 50% 1-year survival when treated with azathioprine, prednisolone, and cyclosporine (see [Chapter 36](#)). Survival is considerably enhanced by a simultaneous bone marrow allograft from the donor animal or by treatment with rabbit antidog thymocyte serum. In practice, median survival times of 8 months can be achieved, with some animals surviving for longer than 5 years. Dogs have significant perioperative mortality, and two thirds experience recurrent acute infections, especially respiratory tract infections with *Bordetella bronchiseptica* and urinary tract infections. Newer immunosuppressive agents such as leflunamide (see [Chapter 36](#)) show promise of vastly improving the prognosis for canine renal allografting.

Cats that receive renal allografts without immunosuppression die in 8 to 34 days. Immunosuppressive therapy involves the use of prednisolone and cyclosporine possibly supplemented with ketoconazole. (The ketoconazole suppresses cyclosporine meta-bolism in the liver and significantly prolongs its half-life.) The therapy can begin 2

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days before surgery so that cyclosporine levels are optimal when the graft is introduced. Six-month survival of treated cats ranges from 59% to 70%, whereas 3-year survival ranges from 40% to 50%. The longest survival time reported for cats receiving renal allografts is 81 months. These figures are gradually improving as experience grows. Long-term complications include acute or chronic rejection and opportunistic infections. (Infection is the second-most important cause of death or euthanasia after acute rejection.) Acute rejection can occur at any time, especially if cyclosporine levels fall below the therapeutic range. Chronic allograft rejection (graft vascular disease) due to progressive arterial arteriosclerosis may cause ischemic graft destruction. It is not responsive to immunosuppressive therapy.

In some circumstances, such as when a dog has maintained functioning renal allografts for several years, immunosuppressive therapy may be reduced gradually and eventually discontinued as graft acceptance becomes complete. It is probable that the immunosuppressive drugs gradually eliminate antigen-sensitive cells. Once their numbers are sufficiently low, the large mass of grafted tissue may be sufficient to establish and maintain tolerance.

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29.5 SKIN ALLOGRAFTS

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Although the mechanisms of rejection are similar among different tissues, minor differences are observed in the process. For example, if a skin graft is placed on an animal, it takes several days for blood vessels and lymphatic connections to be established between the graft and the host. Only when these connections are made can host cells enter the graft and commence the rejection process. The first sign of rejection is a transient neutrophil accumulation around the blood vessels at the base of the graft. This is followed by infiltration with mononuclear cells (lymphocytes and macrophages) that eventually extends throughout the grafted skin. The first signs of tissue damage are observed in the capillaries of the graft, whose endothelium is destroyed. As a result, the blood clots, blood flow stops, and tissue death follows rapidly. The presence of Langerhans cells in the epidermis significantly enhances the antigenicity of skin allografts. Skin allograft rejection is delayed if the graft is prevented from developing lymphatic connections with the host. In a second-set reaction, host blood vessels usually do not have time to grow into a skin graft, since a destructive mononuclear cell and neutrophil infiltration rapidly develops in the graft bed.

29.6 LIVER ALLOGRAFTS

It was originally reported that a high percentage of liver allografts between outbred pigs were accepted without immunosuppression. However, these pigs were not genetically defined, and the degree of MHC mismatching was unclear. When liver allografts are made between genetically defined miniature pigs with known MHC differences, it is found that their speed of rejection is similar to that observed with kidney or skin allografts. Liver graft rejection in dogs tends to occur fairly slowly. This inhibition of liver allograft rejection appears to be due to the presence of indoleamine 2,3-dioxygenase (IDO). IDO destroys the amino acid tryptophan. Since tryptophan is essential for Th1 responses, its absence within the grafted liver is highly immunosuppressive.

29.7 CARDIAC ALLOGRAFTS

Acute rejection of canine heart allografts is associated with massive lymphocytic infiltration and myocyte damage leading to rapid graft destruction. If, however, the rejection process is slowed for some reason, the pathologic process in the chronically rejected organ changes. In these cases, lymphocytes and antibodies directed against vascular smooth muscle cells stimulate proliferation. The resulting smooth muscle cell growth and inflammation lead to obliteration of the blood vessel lumen and eventually cardiac failure ([Figure 29-6](#)). This graft

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arteriosclerosis (or graft vascular disease) results from the growth-stimulating effects of both T cell–derived cytokines and antibodies. A similar lesion is seen in renal allografts undergoing chronic rejection. T cells are occasionally but not consistently seen in these lesions.

29.8 CORNEAL ALLOGRAFTS

Certain areas of the body, such as the anterior chamber of the eye, the cornea, the thymus, the testes, and the brain, are immune-privileged sites. As a result, grafts made into these sites may not be rejected. In humans, for example, 90% of first-time corneal allografts survive without tissue typing or immunosuppressive drugs. These sites are privileged because the body rigorously controls inflammation in their critical tissues. Several mechanisms are involved in this. Thus the sites have an impermeable blood-tissue barrier, lack dendritic cells, express low levels of MHC class I and II molecules, and may contain high levels of immunosuppressive molecules such as IDO, transforming growth factor- β (TGF- β) (eyes and testes), neuropeptides (eyes), complement inhibitors (eyes), and corticosteroids (testes). Molecules found in normal aqueous humor also block innate immune mechanisms. Thus they block NK cell lysis, inhibit neutrophil activation by CD95L, suppress nitric oxide production by activated macrophages, and interfere with alternative complement activation. The eye and testes are also unique in that they express very high levels of CD95L (fas ligand). As a result, any CD95⁺ (fas)-activated T cells that enter these organs will bind to CD95L and be killed by apoptosis.

29.9 BONE ALLOGRAFTS

Bone cortical allografts are used to repair severe nonreconstructible diaphyseal fractures as well as to reconstruct defects created by the resection of tumors. Rejection of bone allografts is rarely a problem, probably because of the absence of soft tissues in the graft. Unfortunately, long-term bone allografts have a high incidence of mechanical failure because the graft is resorbed before it is replaced. Joints may be successfully transplanted in horses provided that such joints have been previously frozen.

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FIGURE 29-6 A section of coronary artery from a canine cardiac allograft. There is severe narrowing of the lumen as a result of smooth muscle cell proliferation, an example of graft vascular disease. Original magnification $\times 100$. (From Penn OC, McDicken I, Leicher F, Bos E: *Transplantation* 22:313, 1976.)

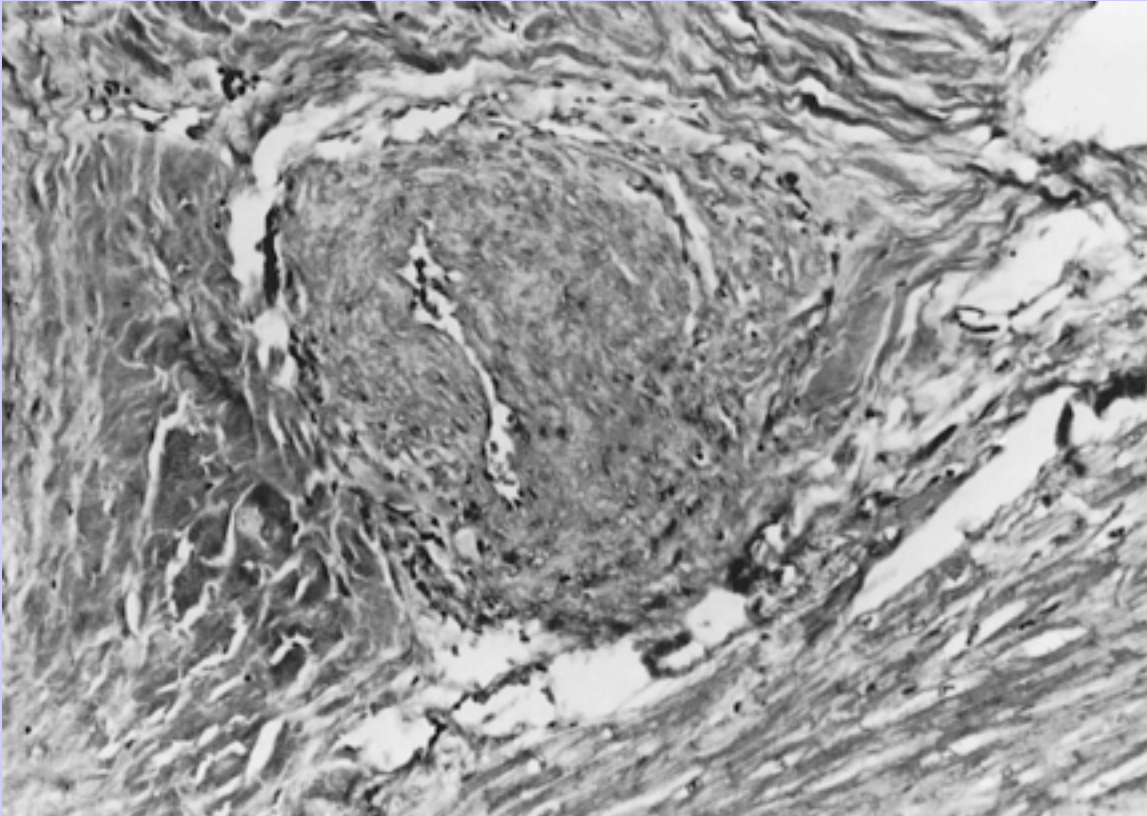
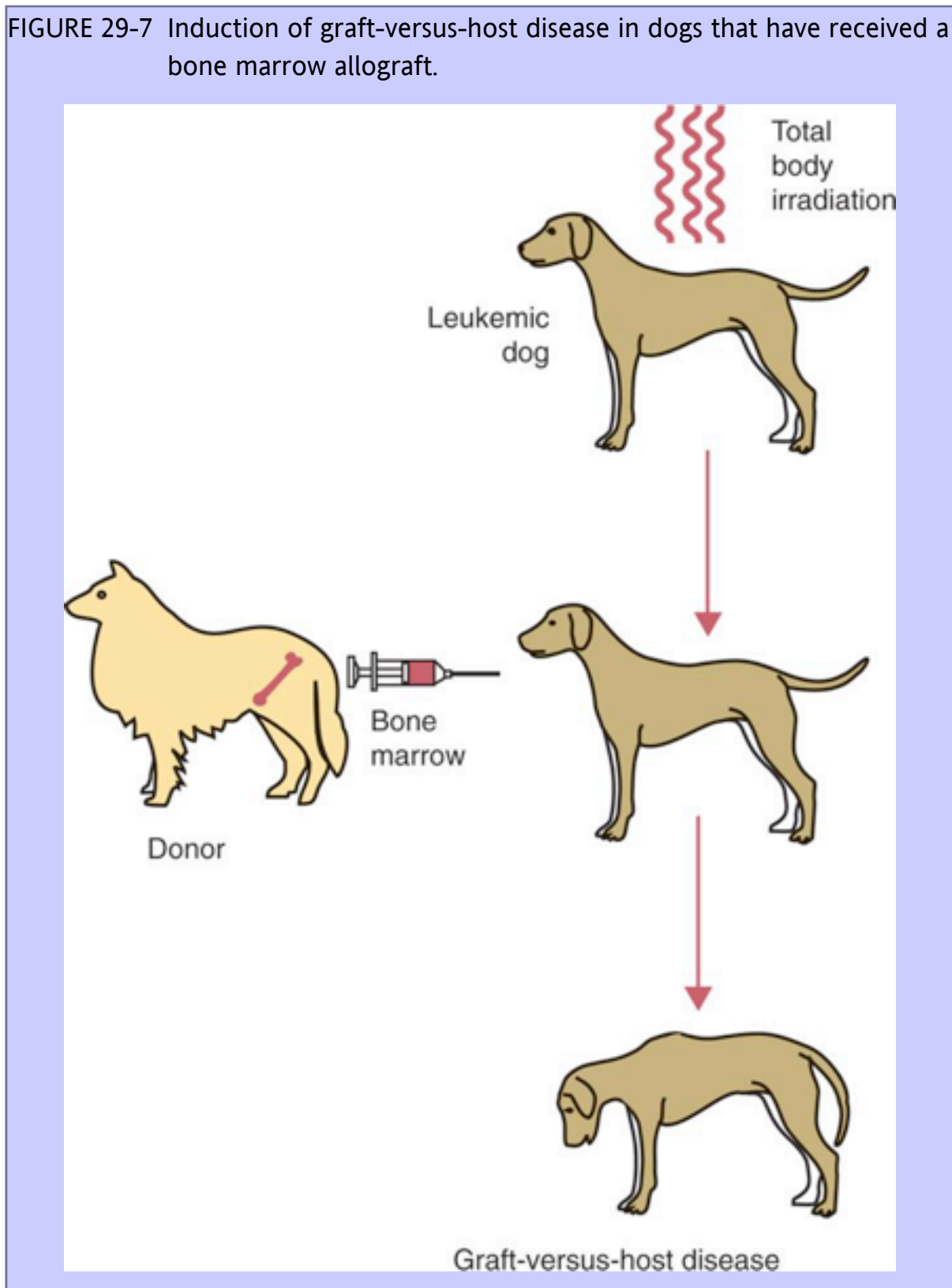


FIGURE 29-7 Induction of graft-versus-host disease in dogs that have received a bone marrow allograft.



29.10 BONE MARROW ALLOGRAFTS

High dose, total-body irradiation may be administered to dogs in order to completely destroy tumors such as leukemias. Unfortunately, such treatment also destroys bone marrow stem cells. These must therefore be replaced by a bone marrow allograft. The new hematopoietic stem cells restore bone marrow function ([Figure 29-7](#)). The recipient dog must first be conditioned by total-body irradiation or chemotherapy with cyclophosphamide. This creates space for the growing transplanted cells, reduces the intensity of the rejection process, and, in leukemic animals, destroys all tumor cells. Marrow is aspirated from the long bones of the donor and administered intravenously to the recipient. Hematopoietic stem cells migrate from the blood to the bone marrow. An optimal dose is about 2×10^8 allogeneic bone marrow cells per kilogram of body weight for matched recipients. The success rate of this procedure is relatively low. Thus there was a 20% success with untreated mismatched canine marrow allografts but a 90% success with treated matched allografts. In a successfully engrafted dog it takes about 30 days for the granulocytes to return to normal, but the lymphocytes take about 200 days to recover. Marrow survival is not generally enhanced by treatment with single immunosuppressive agents, but combinations such as mycophenolate mofetil plus cyclosporine or methotrexate plus cyclosporine can result in the development of stable canine bone marrow chimeras.

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FIGURE 29-8 Very severe cutaneous erythematous lesions on the face of a dog suffering from graft-versus-host disease as a result of a bone marrow allograft. (From Harris CK, Beck ER, Gasper PW: *Compend Contin Educ Prac Vet* 8:337, 1986.)



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29.10.1 Graft-versus-Host Disease

If healthy lymphocytes are injected into the skin of an allogeneic recipient, the lymphocytes attack the host cells and cause local acute inflammation. Provided the recipient has a functioning immune system, this graft-versus-host (GVH) reaction is not serious because the recipient is able to destroy the foreign lymphocytes and thus terminate the reaction. If, however, the recipient cannot reject the grafted lymphocytes because it has been immunosuppressed or is otherwise immunodeficient, these grafted cells may cause uncontrolled destruction of the host's tissues and, eventually, death. Thus GVH disease occurs in bone marrow allograft recipients who have been effectively immunosuppressed by total-body irradiation or cyclophosphamide treatment.

The lesions generated in GVH disease depend on the MHC disparity between donor and recipient. When they differ only in the MHC class I molecules, the disease is caused by cytotoxic T cells that attack all the nucleated cells of the host. This leads to a wasting syndrome characterized by bone marrow destruction leading to pancytopenia, aplastic anemia, loss of T and B cells, and hypogammaglobulinemia. Lymphocytes infiltrate the intestine, skin, and liver and secrete TNF- α , which causes mucosal destruction and diarrhea, skin and mouth ulcers, liver destruction, and jaundice. (Th1 cytokines are produced in GVH disease, and it can be treated by antibodies to IFN- γ or TNF- α .)

If there is only an MHC class II disparity between the donor and recipient, both graft and host CD4⁺ T helper cells may be stimulated. The production of Th2-derived cytokines may lead to immunostimulation, autoantibody formation, and even a syndrome resembling systemic lupus erythematosus and polyarthritis (see [Chapter 33](#)). (This is called autoimmune GVH disease and may be treated with antibodies against IL-4.)

In practice, pure class I or class II disparities rarely occur naturally. Thus in dogs GVH disease can either be an acute disease causing death within 4 weeks of transplantation or it can be prolonged and chronic. The major target organs are the skin, liver, gastrointestinal tract, and lymphoid system. The first clinical signs are exudative ear lesions, scleral injection, hyperkeratosis, alopecia, skin atrophy, and generalized erythema seen by 10 days ([Figure 29-8](#)). Jaundice and diarrhea frequently occur, as does inflammation of the eyes, nose, and oral mucous membranes. An antiglobulin-positive hemolytic anemia may also occur. The immunosuppressive drug methotrexate, together with monoclonal antilymphocyte antibodies, may be used to suppress GVH disease.

It is of interest to note that bone marrow transplantation in cats that previously received immunosuppressive X-radiation or cyclosporine is a very successful procedure, and GVH disease is not a major problem in this species.

29.11 XENOGRAFTS

Although humans currently receive organs from dead human donors, the demand for organ grafts greatly exceeds the supply. It is possible that xenografting from nonhuman donors would eliminate this shortage. Unfortunately, xenografts are usually rejected within a few hours. The pathology of hyperacute xenograft rejection includes extensive hemorrhage and thrombosis brought about by massive destruction of endothelial cells. This allows blood cells to escape while at the same time exposing the underlying basement membrane to platelets

Concordant xenografts are those between two closely related species, such as between a chimpanzee and a human. 388

In these cases rejection is largely mediated by cellular reactions. In discordant xenografts (those between unrelated species such as from a pig to a human), rejection is mediated largely by humoral mechanisms. In practice, concordant human xenografting from other primates such as chimpanzees or baboons is impractical because of the difficulty in providing large numbers of donor animals. Pigs, however, may be more practical sources of organs. 389

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They breed rapidly and their organs are of an appropriate size. Unfortunately pig organs trigger a severe discordant xenograft rejection response in humans mediated by natural anticarbohydrate antibodies. Humans and Old World monkeys lack the enzyme α 1-3 galactosyltransferase and do not therefore make carbohydrates or glycoproteins with the α 1-3galactosyl linkage. Because humans are exposed to this structure on many bacteria, we make high levels of antibodies to the Gal α 1-3Gal epitope. Indeed more than 2% of total human immunoglobulin M (IgM) and IgG consists of antibodies to this α Gal epitope. α Gal, on the other hand, is a major component of pig glycoproteins. Thus if a pig organ is grafted into a human, these antibodies bind to graft cells, activate the classical complement pathway, and induce rapid cell lysis. A second mechanism that contributes to hyperacute rejection is activation of the alternate complement pathway. This activation results from the failure of human complement factor H to prevent assembly of the alternate C3 convertase on the surface of pig cells. A third mechanism is the species-specific activity of complement control proteins. Thus the natural inhibitors of complement such as CD46, CD55, and CD59 found on pig cells cannot control the activation of human complement. Transgenic pigs have been produced that express these human complement inhibitors on their cells and do not trigger hyperacute rejection on grafting. Should the xenograft survive attack by these natural antibodies and complement, it is still susceptible to attack from induced antibodies and from antibody-dependent, cell-mediated cytotoxicity mediated through NK cells and monocytes, which together induce a delayed xenograft rejection. Thus many barriers are yet to be overcome if pig organs are ever to be routinely used as human organ transplants.

One other point relevant to xenografting is that donor animals may carry viruses that could cause disease in a severely immunosuppressed recipient or, even worse, recombine with human viruses to create new and potentially hazardous pathogens. These xenograft-derived infections (xenozoonoses) are of special concern if primates are used as organ donors. These animals are known to carry viruses such as simian immunodeficiency virus and herpes B virus that can infect humans. Pigs possess an endogenous retrovirus that has the ability to infect some human cell lines in tissue culture, although it is not known to cause human disease.

29.12 ALLOGRAFTS AND THE REPRODUCTIVE SYSTEM

29.12.1 Sperm

Allogeneic sperm can successfully and repeatedly penetrate the female reproductive tract without provoking a significant immune response. The reason for this is that seminal plasma is immunosuppressive. Sperm exposed to this fluid are nonimmunogenic, even after washing. Prostatic fluid, one of the immunosuppressive components of seminal plasma, also inhibits complement-mediated hemolysis. In cattle, the immunosuppressive components of seminal plasma are proteins of less than 50 kDa and 150 kDa. Nevertheless, occasional cases of infertility resulting from the production of antisperm antibodies in the uterus do occur.

29.12.2 Pregnancy

When mammals evolved to become viviparous and the fetus developed inside its mother, a significant immunological problem had to be overcome. The fetus could not be rejected like an allograft even though it possesses paternal MHC molecules and its trophoblast lodges deep in the uterine wall. In a normal pregnancy the fetus establishes and maintains itself in spite of these MHC differences. The uterus is not a privileged site, since grafts of other tissues, such as skin, in the uterine wall are readily rejected. Likewise, a mother may make antibodies against fetal blood group antigens and these can destroy fetal red blood cells either in utero, as in primates, or following ingestion of colostrum, as occurs in other mammals (see [Chapter 26](#)). Nevertheless, allograft rejection does not occur.

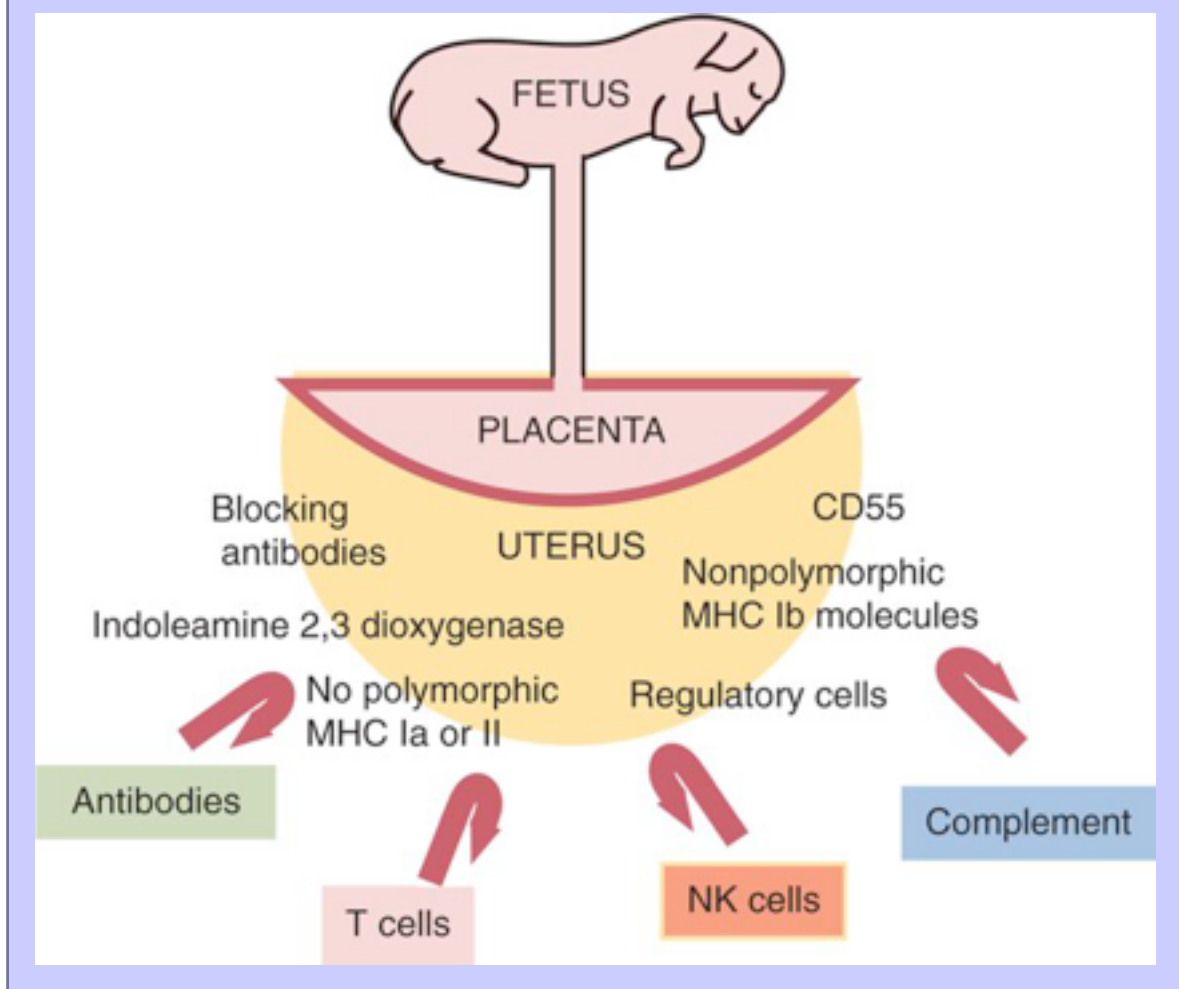
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We know that a pregnant mother may mount an immune response to her fetus. For example, in the pregnant mare, placental cells invade the uterine wall to form structures called endometrial cups. These in turn stimulate a strong immune response by the mare against paternal MHC antigens around day 60 of gestation. As a result, the cups are surrounded by large numbers of $CD4^+$ and $CD8^+$ lymphocytes, macrophages, and plasma cells. Virtually all mares carrying an MHC-incompatible fetus produce strong antibody responses to paternal MHC class I antigens by day 60 of pregnancy. This eventually leads to degeneration of the endometrial cups around 120 days of pregnancy. Despite these responses, the pregnancy is unaffected. It is possible that these interactions lead to a Th2

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FIGURE 29-9 Some of the immunosuppressive factors that prevent rejection of the fetus by the mother's immune system.



immune response dominated by IL-10 production that does not threaten the pregnancy rather than a Th1 response that could lead to fetal rejection. In general, pregnancy is associated with a strong skewing of the mother's immune system in favor of Th2 responses and a reduction in Th1 responses. (This raises the interesting concept that infections that promote a strong Th1 response might reverse this skewing, compromise pregnancy, and lead to abortion. This would certainly apply to protozoan infections such as toxoplasmosis, *Neospora caninum* infection, and brucellosis.)

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The immunological destruction of the fetus and its trophoblast is prevented by the combined activities of many different immunosuppressive mechanisms acting at the maternal-fetal interface (Figure 29-9). First, no polymorphic MHC molecules are expressed on preimplantation embryos or oocytes, and these cannot be induced by exposure to interferon. Likewise, MHC class Ia or class II molecules are not expressed on the cell layer of the trophoblast in contact with maternal tissues. Cytokines that usually enhance MHC expression, such as IFN- γ , have no effect on trophoblast cells. The absence of MHC should permit trophoblast cells to avoid destruction by cytotoxic T cells but make them susceptible to attack by NK cells. This is prevented, however, by expression of the nonpolymorphic class Ib molecules, human leukocyte antigen (HLA) G and E. These molecules prevent attack on the trophoblast by NK cells while the NK cells control invasion of the uterine wall by the trophoblast. Thus there is a balance between MHC class Ib expression on the trophoblast and NK cells in the uterine wall that regulates trophoblast growth and invasion. NK cells found in the placenta are of a distinct type called endometrial gland cells (uNK cells). uNK cells have been described in rodents, bats, and horses. They invade the pregnant porcine uterus in large numbers.

CD4⁺ CD25⁺ T_{reg} cells increase during pregnancy and appear to play an important role in preventing fetal rejection. Estrogen treatment and pregnancy both induce FoxP3 protein and may help support this regulation. It has been demonstrated that trophoblast cells secrete IDO, which blocks Th1 responses and promotes apoptosis. Inhibitors of IDO permit maternal rejection of allogeneic fetuses in mice. T_{reg} cells upregulate IDO expression in dendritic cells. In addition, IDO induces trophoblast HLA-G expression, suggesting that these molecules interact in order to maintain pregnancy.

Although the trophoblast minimizes maternal sensitization by allogeneic fetal cells, cytotoxic T cells or antibodies can develop during pregnancy. For example, up to 90% of pregnant mares make antibodies to foal MHC class I molecules. Similar antibodies frequently develop in multiparous sheep and cattle. In some mouse strains, up to 95% of pregnant animals make antibodies against fetal MHC molecules. Up to 40% of women make antibodies to fetal MHC molecules after giving birth. The presence of these antibodies has no adverse effect on the course of the pregnancy. On the contrary, the maternal immune response may actually stimulate placental function. Thus in mice, hybrid placentas are larger than the placentas of inbred animals, and females tolerant to paternal antigens have smaller placentas than intolerant females. Other studies show that mothers sensitized to paternal MHC molecules have better fetal survival. This effect may be due to the stimulatory effect of IL-3 and granulocyte-macrophage colony-stimulating factor from maternal T cells on trophoblast growth. It is of interest to note that in cattle there is a clear association between retention of the placenta and its MHC class I molecules. In general, MHC class I compatibility between a mother and her calf increases the risk of a retained placenta. MHC class II compatibility had no effect. It has been suggested that the expulsion of the placenta after birth may be due, at least in part, to an allograft response.

Some antibodies made by the mother against fetal antigens may coat placental cells, thus preventing their destruction by maternal T cells. This blocking antibody can be eluted from the placenta and has been shown to suppress other cell-mediated immune reactions against paternal antigens, such as graft rejection. Absence of this blocking antibody accounts for some cases of recurrent abortion in women. Nevertheless it can also be shown that totally immunodeficient mice can have successful pregnancies. Mouse trophoblast cells are protected against complement-mediated damage by high levels of expression of a complement inhibitory protein called Crry on their surface. In its absence, fetal rejection appears inevitable. CD55 (DAF) is incorporated in the trophoblast at the fetomaternal interface and so protects it against complement attack.

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The fetus does not depend entirely on maternal mechanisms for its protection. The placenta is a source of many immunosuppressive factors, including estradiol and progesterone and possibly also chorionic gonadotropin.

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Some isoforms of α -fetoprotein, the major protein in fetal serum, are immunoregulatory. In addition, some pregnancy-associated glycoproteins, including α_2 -macroglobulin, a molecule related to TGF- β (possibly derived from T_{reg} cells infiltrating the placenta) and placental interferons, have immunosuppressive properties. In mammals, unique interferons (IFN-w in humans, horses, and dogs; IFN-t in ruminants; and IFN- γ and IFN- δ in pigs) from the embryonic trophoblast act as signaling proteins between the embryo and mother during early development. These interferons can also inhibit lymphocyte proliferation. Amniotic fluid is rich in immunosuppressive phospholipids. Despite the previous discussion, if the antigenic differences between the mother and her fetus are very great, pregnancy may not go to completion. Thus studies on xenogeneic combinations of two mouse species show that the embryos develop until midgestation and are then attacked and destroyed by maternal lymphocytes. Similarly, donkey embryos transferred to horse mares are destroyed by large numbers of maternal lymphocytes.

Mild immunosuppression is a consistent feature of late pregnancy and the early postpartum period. Pregnant animals may have minor deficiencies in cell-mediated immune reactivity to nonfetal antigens. Dairy cows experience a periparturient depression in neutrophil function and reduced T cell cytotoxicity and cytokine production. This suppression appears to be mediated by CD8⁺ T cells. In mares, blood lymphocyte responses to mitogens drop from 4 weeks before to 5 weeks after parturition. NK cell activity in pigs drops at the end of gestation to reach a low point 2 to 3 weeks after parturition. Ewes in late pregnancy may show a reduction of some immunoglobulin classes such as IgG1. This may be due to alterations in T helper cell function or, more plausibly, to diversion of the IgG1 into the mammary gland to produce colostrum. This suppression may be significant in parasitized animals, where the immune response barely controls the parasite. Similarly, immunosuppression may permit *Demodex* mite populations to rise in pregnant or lactating bitches and aid in the transmission of mites to their puppies.

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30 CHAPTER 30 Resistance to Tumors

30.1 KEY POINTS

- Under some circumstances cancer cells may be antigenically different from normal body cells. As a result they may trigger an immune response and be rejected. This immune response may not, however, be very strong.
- Because cancer cells can mutate as they grow, they may be selected for their lack of antigenicity.
- The most important mechanism of destruction of spontaneous tumors probably involves killing by natural killer (NK) cells. NK cells recognize and attack target cells that fail to express major histocompatibility complex class I molecules.
- Under some circumstances cytotoxic T cells, activated macrophages, or antibodies may also attack cancer cells.
- Failure of antitumor immunity not only involves tumor cell selection but also regulatory T cells and blocking antibodies.
- It has proved very difficult to devise effective, consistent antitumor immunotherapy. Successful antitumor immunotherapy may involve administration of cytokines or antibodies or active immunization involving antitumor vaccines.
- Many tumors are profoundly immunosuppressive.

Normal body processes depend on careful regulation of cell division. When cells multiply, it is essential that they do so only as and when required. Unfortunately, as a result of mutations triggered by chemicals, radiation, or virus infection, cells may occasionally break free of the constraints that regulate cell division. A cell that is proliferating in an uncontrolled fashion will give rise to a growing clone of cells that eventually develops into a tumor or neoplasm. If these cells remain clustered together at a single site, the tumor is said to be benign. Benign tumors can usually be removed by surgery. In some cases, however, tumor cells break off from the main tumor mass and are carried by the blood or lymph to distant sites, where they lodge and continue to grow. This form of tumor is said to be malignant. The secondary tumors that arise in these distant sites are called metastases. Treatment for malignant tumors may be very difficult because it may be impossible to remove all metastases surgically. Malignant tumors are subdivided according to their tissue of origin. Tumors arising from epithelial cells are called carcinomas; those arising from mesenchymal cells, such as muscle, lymphoid, or connective tissue cells, are called sarcomas. A leukemia is a tumor derived from hematopoietic stem cells.

The essential difference between a normal cell and a tumor cell is a loss of control of cell growth as a result of multiple mutations. These mutations may also result in the tumor cells' expressing abnormal proteins on their surface. These proteins may, under some circumstances, be recognized by the immune system and so trigger immunological attack.

30.2 TUMORS AS ALLOGRAFTS

When organ transplantation became a common procedure as a result of the development of potent immunosuppressive drugs, it was found that patients with prolonged graft survival were many times more likely to develop certain cancers than were nonimmunosuppressed individuals. It was also observed that some immunodeficient patients had an increased tendency to develop some malignant tumors. For example, patients with acquired immune deficiency syndrome (AIDS) may develop Kaposi's sarcoma. It was therefore suggested that the immune system was responsible for the prevention of cancer. From this suggestion the immune surveillance theory emerged. This theory held that the body constantly produces neoplastic cells but that in a healthy individual the immune system rapidly recognizes and eliminates these cells through cell-mediated mechanisms. The theory suggested that progressive cancer would result if the cancer cells somehow evaded recognition by T cells.

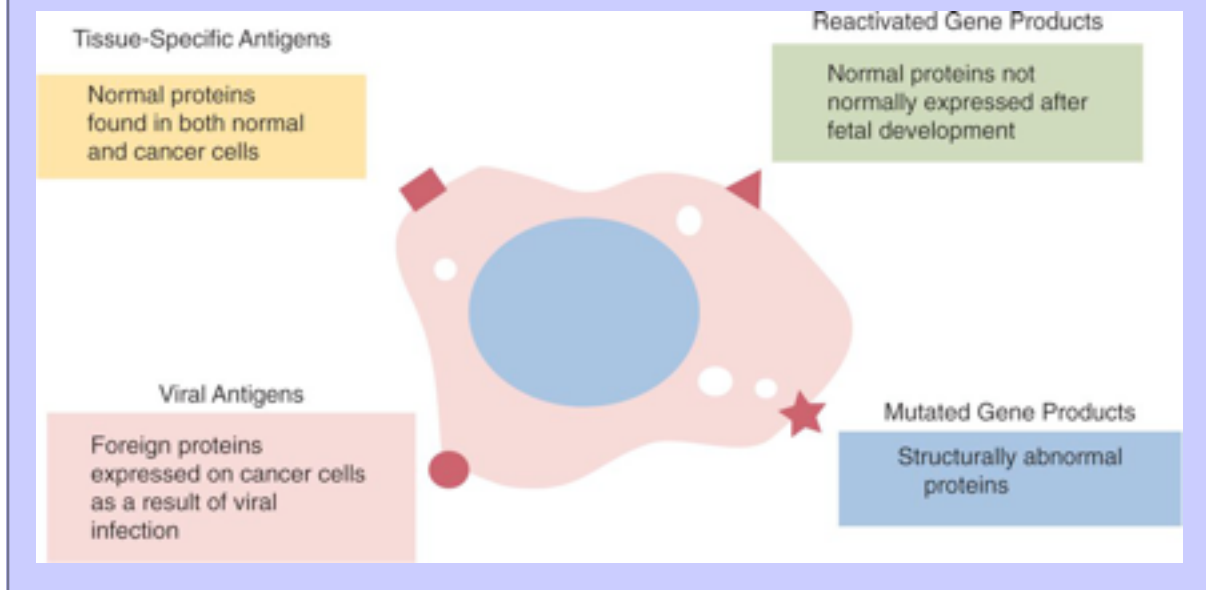
The immune surveillance theory soon ran into problems. Common human cancers such as those of the lung or breast did not develop more frequently in immunodeficient people. Likewise nude (*nu/nu*) mice, although deficient in T cells, are no more susceptible than normal mice to chemically induced or spontaneous tumors (see [Chapter 34](#)). Finally, by using transgenic mice in which specific T cells for tumor-associated antigens were carefully monitored, it became clear that many tumor antigens induce tolerance in a manner similar to normal self-antigens. Thus most evidence has supported the idea that the immune system does not normally distinguish between tumor cells and normal, healthy cells.

Notwithstanding this, there are some situations where the immune system appears to recognize and kill cancer cells. For example, some immunodeficient mouse strains, which are “cleaner” subjects than nude mice, show an increased prevalence of spontaneous cancer. (Nude mice have some persistent T and B cell function and intact innate defenses.) These include recombination-activating gene (RAG) knockout mice that cannot produce functional T or B cells and signal transducer and activator of transcription 1 (STAT-1) knockout mice that lack both acquired and innate responses by being unresponsive to interferon- γ (IFN- γ). RAG knockout mice suffer from an increased incidence of spontaneous tumors of the intestinal epithelium, whereas RAG/STAT-1 knockout mice develop mammary cancers.

It is therefore perhaps more appropriate to think of the immune system as “immunoediting” cancers. Thus under some circumstances the immune system can indeed eliminate cancer cells in a manner consistent with immunosurveillance. If however the cancer cells can evade destruction, they may survive indefinitely or be selected by the host's immune responses to produce new populations of tumor cell variants. Eventually, however, some of these variants may evade the control of the immune system entirely and grow to produce clinical cancer.

Whether cancer cells are eliminated by immune mechanisms is probably determined in part by whether they also cause inflammation. Thus if a metastasizing tumor does not invade lymphoid organs, it may escape immune surveillance. On the other hand, tumors that invade lymph nodes can be divided into strongly and weakly immunogenic types. Strongly immunogenic tumors elicit a strong T cell response following processing by dendritic cells. Weakly immunogenic tumors tend to grow as walled-off nodules that may not be processed in sufficient amounts to trigger immune responses. These are the commonest tumors in humans. It is possible that tumor cells that trigger inflammation in tissues trigger dendritic cell activation and processing. On the other hand, tumors that fail to generate inflammation may simply be ignored by the immune system. Alternatively, it is possible that the antigens of the tumor may be tolerated by the immune system. If successful tumor therapy is to be achieved, then these two states must be distinguished.

FIGURE 30-1 Some of the great variety of new antigens that may appear on the surface of tumor cells and provoke an immune response.



Most humans that develop “spontaneous” cancer have normal immune systems. On the other hand, immunosuppressed individuals such as allograft recipients and AIDS victims develop a very different spectrum of cancers from that of the general population. The only cancers to which they are at greater risk are those caused by viruses, such as Kaposi's sarcoma. Immunosuppressed individuals are no more likely than the general population to develop the common cancers, such as those of breast, lung, or colon.

Although the original surveillance hypothesis has had therefore to be drastically modified, it is clear that under some circumstances the immune system may destroy tumor cells and that this response may be enhanced to protect an individual against some cancers. However, there is a great difference between the strong and effective cell-mediated immune response triggered by foreign organ grafts and the much weaker responses to the antigens associated with tumor cells.

30.2.1 Tumor Antigens

In order to distinguish between normal and tumor cells, the host's T cells must recognize tumor cell antigens. Five major types of tumor antigen have been identified. The first type is composed of differentiation antigens associated with specific stages in the development of a cell type. For example, some tumor cells may express the products of developmental genes that are turned off in adult cells and are normally only expressed early in an individual's development. These proteins are called oncofetal antigens. Examples include tumors of the gastrointestinal tract that produce a glycoprotein called carcinoembryonic antigen (CEA; also called CD66e), normally found only in the fetal intestine. The presence of detectable amounts of CEA in serum may therefore indicate the presence of a colon or rectal adenocarcinoma. α -Fetoprotein produced by hepatoma cells is normally found only in the fetal liver. Likewise, squamous cell carcinoma cells may possess antigens normally restricted to fetal liver and skin. These oncofetal antigens are usually poor immunogens and do not provoke protective

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immunity. However, their measurement in blood may be useful for diagnosis and for monitoring the progress of the tumor.

The second type includes mutated forms of normal cellular proteins. For example, melanoma cells may express the products of mutated oncogenes on their surface ([Figure 30-1](#)). Some tumor antigens are recognized because they are abnormally glycosylated. Chemically induced tumors may express mutated surface antigens unique to the tumor and not to the inducing chemical ([Figure 30-2](#)). Because carcinogenic chemicals can produce many different mutations, tumors induced by a single chemical in different animals may be antigenically different. Even within a single chemically induced tumor mass, antigenically distinct subpopulations of cells exist. As a result, immunity to one chemically induced tumor does not prevent growth of a second tumor induced by the same chemical.

The third type is made up of normal proteins produced in excessive amounts. A good example is the production of prostate-specific antigen (PSA) in prostate carcinomas of humans. PSA is a protease exclusively produced by the prostate epithelium. Increased blood levels of this protein indicate excessive prostate activity. One cause of this is the growth of a carcinoma.

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FIGURE 30-2 Although oncogenic viruses induce tumor cells with identical new antigens, chemical carcinogens induce tumor cells with a great variety of new antigens.

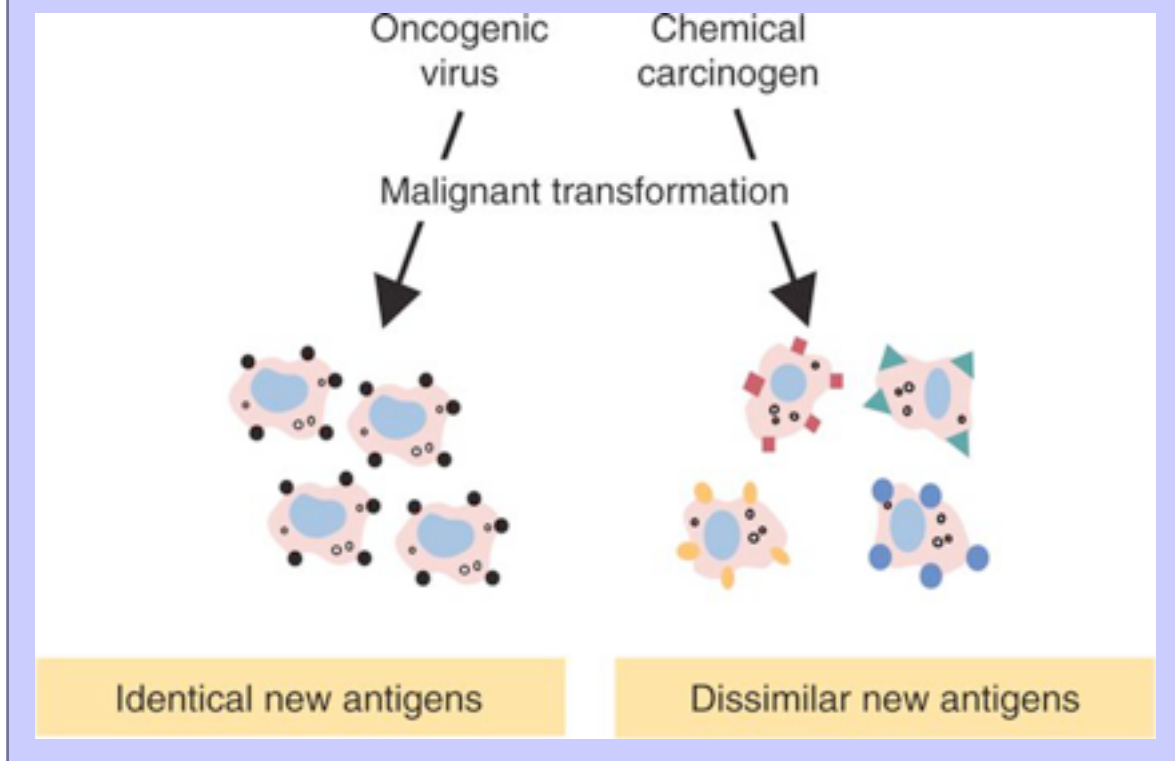
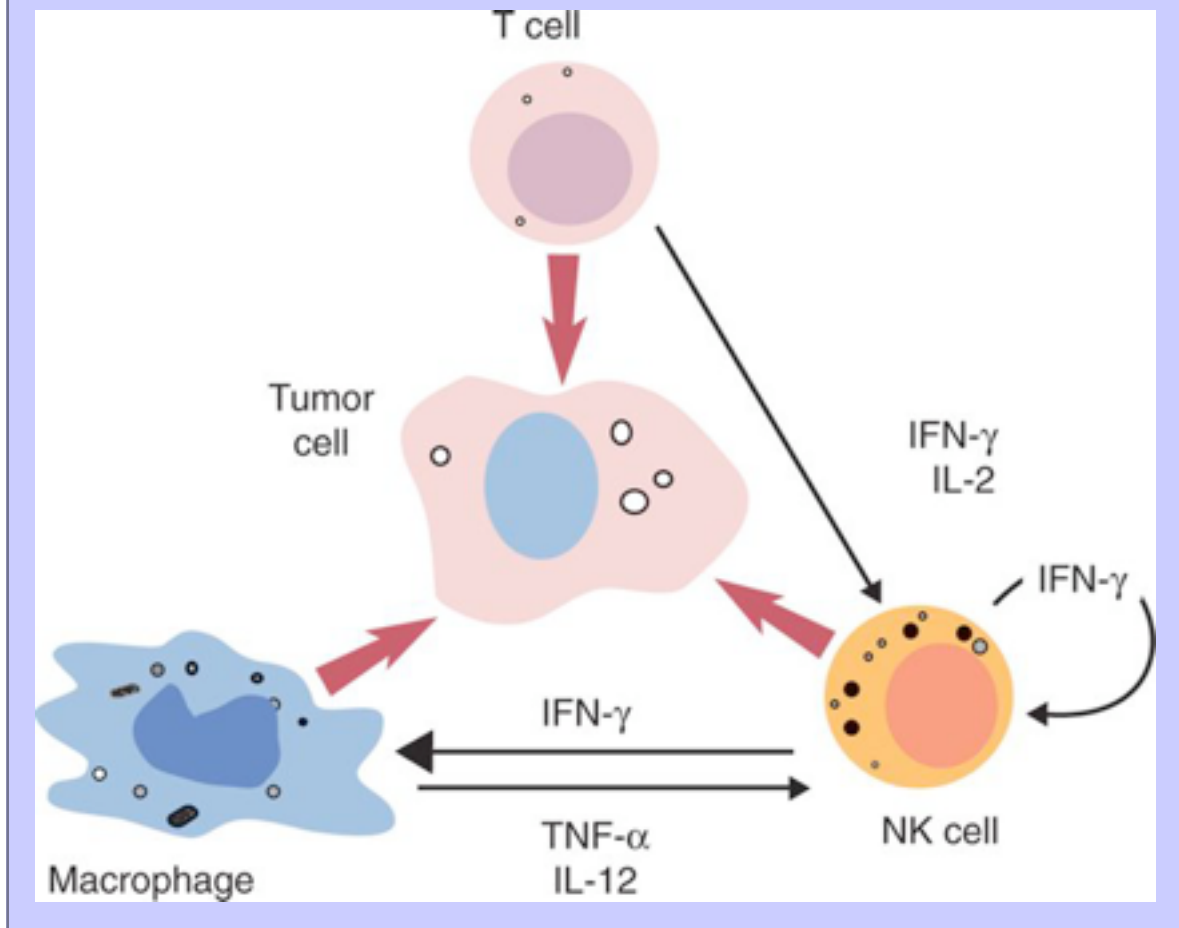


FIGURE 30-3 The three major cell types that participate in tumor cell destruction and the role of interferon in stimulating that activity.



The fourth type is cancer/testis (CT) antigens, a group of tumor antigens with gene expression restricted to male germ cells in the testis and to various malignancies. Their function in tumors is unknown.

The fifth comprises tumors induced by oncogenic viruses, which tend to gain new antigens characteristic of the inducing virus or of other endogenous retroviruses. These antigens, although coded for by a viral genome, are not part of a virion. Examples include the feline oncornavirus cell membrane antigens found on the neoplastic lymphoid cells of cats infected with feline leukemia virus and Marek's tumor-specific antigens found on Marek's disease tumor cells in chickens. (Both of these are virus-induced, naturally occurring, T cell tumors.)

30.3 IMMUNITY TO TUMORS

If tumor cells express different antigens from normal cells, then why are they not regarded as foreign and attacked by the immune system? The main problem appears to be that the abnormal molecules are not appropriately presented to the cells of the immune system, especially cytotoxic T cells. Nevertheless on occasion tumor cells may be attacked by natural killer (NK) cells, cytotoxic T cells, activated macrophages, or antibodies ([Figure 30-3](#)). It is likely that the most important of these are NK cells.

30.4 NATURAL KILLER CELLS

About 15% of mammalian blood lymphocytes are neither T nor B cells but belong to a third population called NK cells. Unlike T and B cells that circulate as resting cells and require several days in order to be activated, NK cells can be activated almost immediately by interferons from virus-infected cells and by interleukin-12 (IL-12) from macrophages. As a result, NK cells enter tissues and rapidly attack tumors and virus-infected cells. They can participate in innate defenses long before antigen-specific T or B cells are generated. Unlike T and B cells, NK cells do not rearrange T cell antigen receptor (TCR) or immunoglobulin genes to produce a repertoire of antigen receptors. Indeed, they do not express antigen-specific receptors at all. Instead they use different combinations of receptors to bind to abnormal cells. By using multiple receptors in this way, NK cells can discriminate between normal and abnormal cells.

NK cells in most mammals are large, granular, nonphagocytic lymphocytes ([Figure 30-4](#)). They probably arise from the same bone marrow precursor as T cells. However, T cells depend on thymic processing for their full development whereas NK cells do not. NK cells are concentrated in peripheral blood, lymph nodes, spleen, and bone marrow. None is found in the thymus.

30.4.1 Surface Markers

NK cells do not express conventional antigen receptors like B cell antigen receptors or TCRs, nor do they express a CD3 complex. On the other hand, a major surface antigen called CD56 is restricted to NK cells. They also express CD2, CD16 (FcγRIII), CD178 (CD95L, or fas ligand), CD40L (CD154), toll-like receptor 3 (TLR3), and TLR9. NK1.1 is an activation receptor restricted to mouse NK cells. There are probably subsets of NK cells since they express varying amounts of CD56 while not all express CD16.

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FIGURE 30-4 A transmission electron micrograph of a human natural killer cell. The nucleus is indented and rich in chromatic. The cytoplasm is abundant and contains many granules. Numerous mitochondria, centrioles, and a Golgi are visible. Original magnification $\times 17,000$. (From Carpen O, Virtanen I, Saksela E: *J Immunol* 128:2691, 1982.)

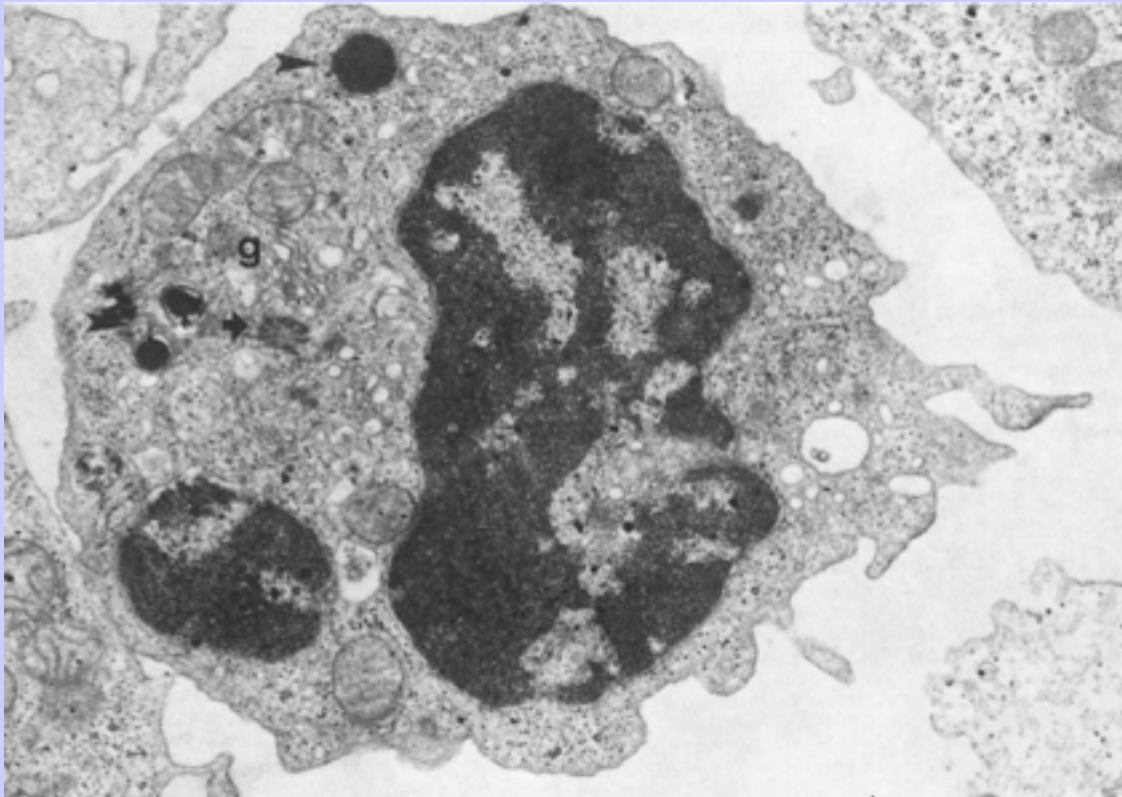
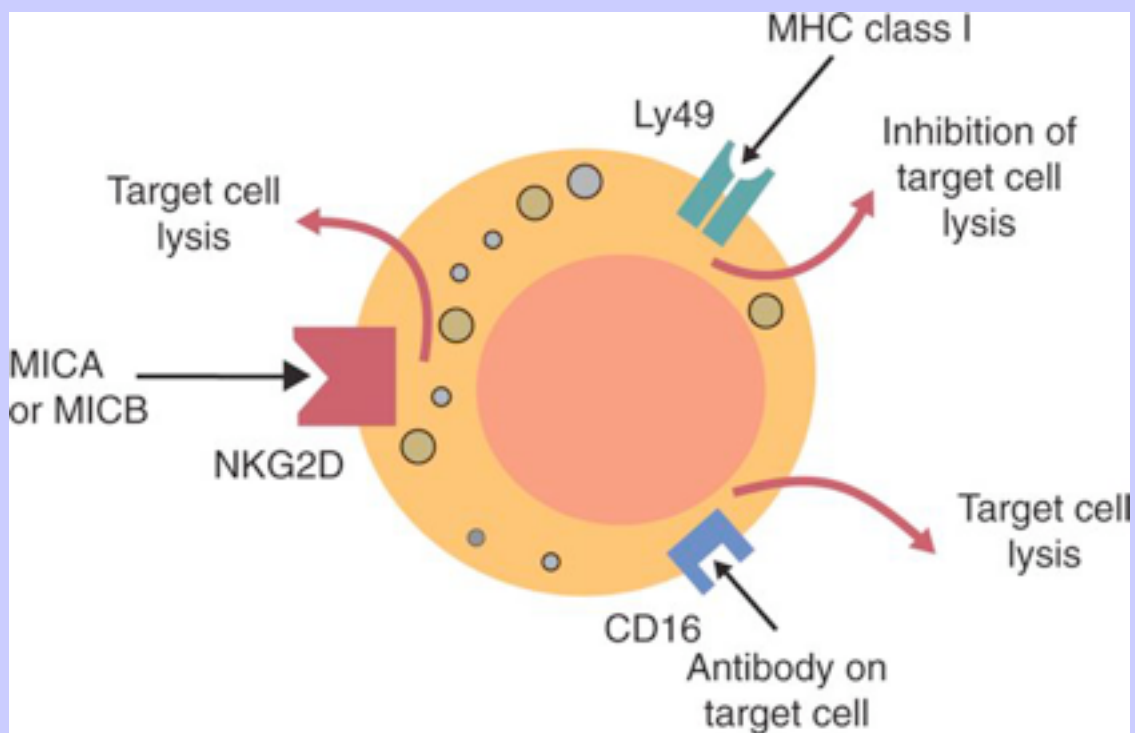


FIGURE 30-5 Three of the receptors found on mouse natural killer (NK) cells. Ly49 recognizes major histocompatibility complex (MHC) class I molecules and suppresses NK cytotoxicity. CD16 binds immunoglobulins and triggers cytotoxicity by antibody-dependent cellular cytotoxicity. NK cells also express NKG2D, a receptor for molecules such as MHC class I chain-related A (MICA) and MICB. These molecules are commonly expressed on tumor cells.



30.4.2 Target Cell Recognition

NK cells recognize and kill abnormal cells using a totally different mechanism than do T cells. NK cell cytotoxicity results from a change in the signals received by activating and inhibitory receptors. When NK cells encounter healthy normal cells, inhibitory signals predominate. When they encounter infected, damaged, or malignant cells, the activating signals prevail. MHC class I molecules expressed on the surface of healthy normal cells provide inhibitory signals sufficient to block NK cell killing. If, however, a target cell does not express even a single MHC class I allele, then the NK will not receive the inhibitory signal and the target cell will be killed ([Figure 30-5](#)). Viruses may suppress MHC class I expression in an attempt to hide within infected cells, and metastatic tumor cells often fail to express MHC class I. These cells are therefore targets for NK cell attack.

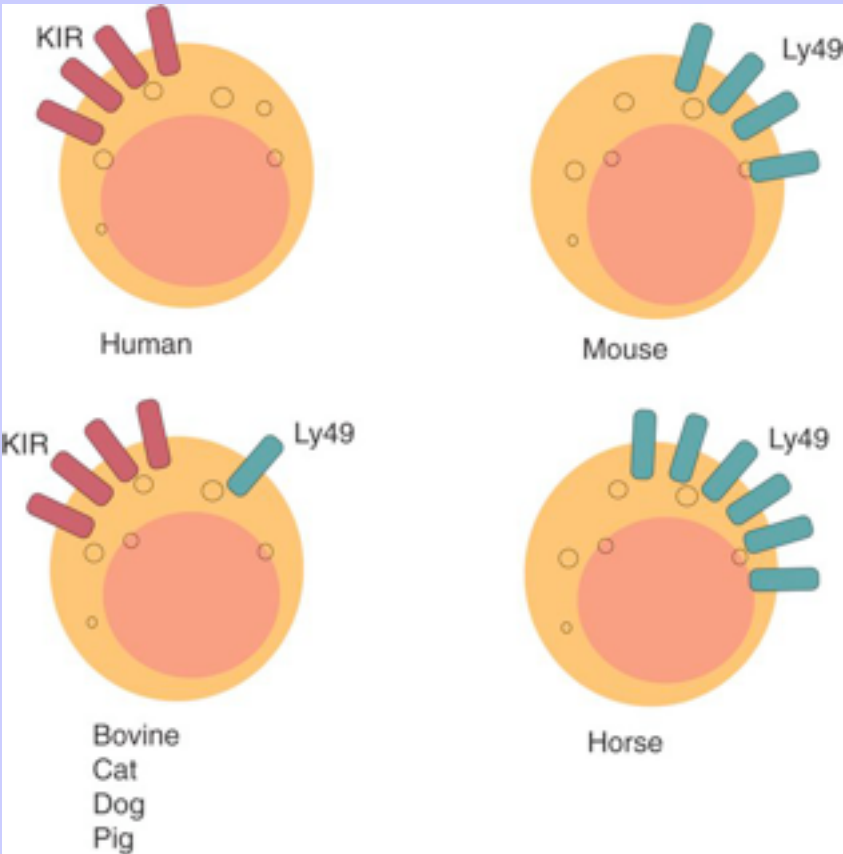
NK cells use surface receptors to identify MHC class I molecules on target cells. These receptors differ between species. In humans NK cell MHC receptors belong to a family of highly polymorphic, killer cell immunoglobulin-like receptor (KIR or CD158) proteins. These are type I transmembrane proteins encoded by a cluster of genes called the leukocyte receptor gene complex. They have varying degrees of polymorphism. Members of the family include the leukocyte immunoglobulin-like receptors (LILRs), the KIRs, and NKp46. NKp46 is only expressed on NK cells, The KIRs are expressed on NK cells and subsets of T cells, and the LILRs are expressed on several types of leukocytes. The KIR and LILR families contain not only cellular-activating receptors but also inhibitors and receptors with no known ligand. Other mammals that employ KIR proteins to bind MHC class I molecules include cattle, pigs, dogs, and cats.

In mice, rats, and horses, in contrast, NK cells identify MHC class I molecules using receptor proteins that are type II transmembrane proteins with a C-terminal

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FIGURE 30-6 The species difference between the major histocompatibility complex (MHC) class I receptors on natural killer cells. Humans possess a single, nonfunctional Ly49 gene. Thus they rely totally on killer cell immunoglobulin-like receptor (*KIR*) molecules, whereas mice rely totally on Ly49 molecules for recognition of their MHC ligands.



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domain similar to that of C-type lectins. They are called killer cell lectin-like receptors (KLRs). They include many different molecules including CD94 and NK1.1. KLRs are encoded by genes located within the NK receptor gene complex. KLRs are highly poly-morphic and multiple copies of their genes are expressed.

Expression of KIRs and KLRs tends to be mutually exclusive. Thus mice do not express KIRs, while humans have only one, nonfunctional KLR gene. Cattle, dogs, cats, and pigs possess multiple KIR genes but only a single functional KLR gene. This suggests that the use of KLR proteins by mice, rats, and horses is not typical of mammals in general (Figure 30-6). However, sequence analysis has also suggested that some bovine KIR molecules may have an inhibitory function whereas others have an opposite effect. The reasons for these species differences are unclear.

A second pathway that triggers NK cell cytotoxicity involves the use of a receptor called NKG2D. This receptor recognizes several proteins expressed by stressed cells. Two of the most important are called MICA (major histocompatibility complex, class I chain-related A) and MICB. These two molecules are expressed when cells are stressed and so are upregulated in tumor cells and virus-infected cells. They are not expressed on normal, healthy cells. When engaged, NKG2D overrides the inhibitory effects of MHC class I molecules and permits the NK cells to kill their target. NKG2D is also expressed on activated γ/δ and α/β T cells. Thus these T cells can also recognize MICA and MICB, suggesting that they too have a role in both acquired and innate immunity. It may be that on surfaces the combination of γ/δ T cells and NK cells kills tumors, while within the body a combination of α/β T cells and NK cells is most effective.

NK cells also recognize target cells by a third, antibody-dependent pathway using CD16. CD16 is an Fc receptor (Fc γ RIII) expressed on NK cells, granulocytes, and macrophages. The form on macrophages and NK cells is a 38-kDa transmembrane protein linked to either the gamma chain of Fc ϵ RI (in macrophages) or the zeta chain (in NK cells). Triggering of NK cells by antigen and antibody through CD16 kills the target cells. NK cells may spontaneously release CD16 so that the NK cell may detach from an antibody-coated target cell after delivering the lethal hit.

30.4.3 Effector Mechanisms

Once triggered, NK cell killing is mediated, as in T cells, through the intrinsic pathway involving perforins and two proteins called granulysin and NK-lysin, as well as through the extrinsic pathway involving CD95L, perforins, granulysin, and NK-lysin constitutively expressed in NK cells. Their expression is increased by exposure to IL-2 and IL-12. The NK cell perforin is a molecule of 70 to 72 kDa (slightly larger than that produced by T cells). It produces characteristic small (5 to 7 nm) lesions in target cell surfaces. Presumably granzymes are injected into the target cells in association with the perforin channels. NK cells also secrete a protease called fragmentin that can induce apoptosis in target cells. Granulysin and NK-lysin can also kill mycobacteria. Because NK cells express TLRs they respond to double-stranded RNA or unmethylated CpG DNA. In the presence of IL-12 or IL-8, stimulation of these TLRs causes release of IFN- γ and tumor necrosis factor- α (TNF- α) and upregulates their cytotoxic ability.

In humans, there is evidence of two subsets of NK cells. NK1 cells produce IFN- γ but almost no IL-4, IL-10, or IL-13. NK2 cells do not secrete IFN- γ but produce IL-13. It is not known whether these subsets occur in domestic mammals.

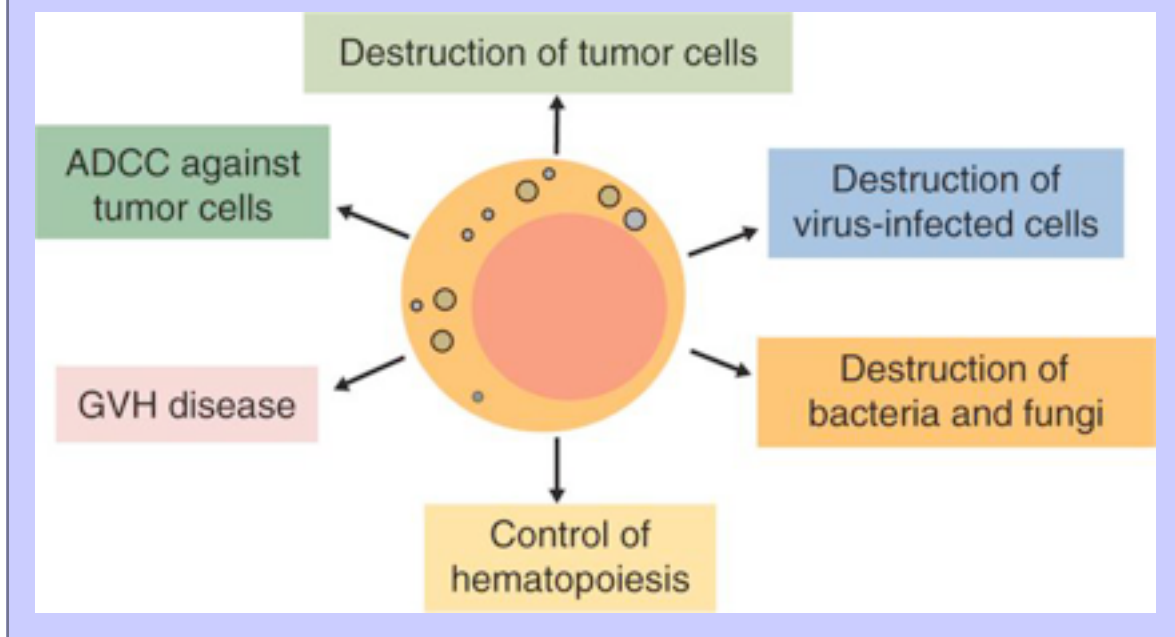
30.4.4 Function

Initially, it was believed that NK cells functioned only as an innate antitumor surveillance system. However, we now recognize that they are active against other targets such as xenografted cells and virus-infected cells ([Figure 30-7](#)). Some KLR molecules on the surface of mouse NK cells can recognize viruses. NK cells can kill bacteria such as *Staphylococcus aureus* and *Salmo*

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FIGURE 30-7 A schematic diagram showing the many functions of natural killer cells.



nella enterica typhimurium, protozoa such as *Neospora caninum*, and some fungi.

Most of the evidence that supports a role for NK cells in innate immunity to tumors is derived from studies on tumor cell lines grown in vitro. Thus NK cells destroy cultured tumor cell lines, and a correlation exists between the level of NK activity measured in vitro and resistance to tumor cells in vivo. It is possible to increase resistance to tumor growth in vivo by passive transfer of NK cells. NK cells destroy human leukemia, myeloma, and some sarcoma and carcinoma cells in vitro, and this activity is enhanced by IFN- γ . NK cells can also invade small primary mouse tumors. Some carcinogenic agents, such as urethane, dimethylbenzanthracene, and low doses of radiation, can inhibit NK activity. Certain stresses, such as surgery, may also depress NK activity and so promote tumor growth.

30.4.5 Regulation

NK cells are activated by cytokines such as IL-1, IL-2, IL-12, IL-15, IL-18, IL-21, and the interferons. For example, culture of NK cells in the presence of IL-2 enhances their cytotoxic activity so that they can lyse normally resistant tumor targets. IL-4 also stimulates NK function and enhances cytotoxicity, whereas IL-3 prevents the death of cultured NK cells. Although NK cells are active in the nonimmunized animal, virus infections or interferon inducers stimulate NK activity above normal levels. These activated NK cells are called

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lymphokine-activated killer (LAK) cells. In these diseases, macrophages phagocytose the invading organisms and produce TNF- α and IL-12. These cytokines induce IFN- γ production by NK cells. This IFN- γ then enhances NK activity by promoting the rapid differentiation of pre-NK cells. It also activates more macrophages so that production of TNF- α is augmented by IFN- γ . The NK cell/interferon system probably has a role in resistance to tumors since neutralization of interferon by specific antisera enhances tumor growth in mice. IFN treatment may be useful in the therapy of some human cancers. For example, IFN- α is of significant benefit in the treatment of hairy cell leukemia (a B cell tumor) and Kaposi's sarcoma in humans. IL-21 from activated CD4⁺ T cells also regulates NK cell function. It blocks activation of uninvolved NK cells, it promotes IFN- γ secretion by activated NK cells, and it initiates apoptosis of activated NK cells while stimulating T and B cell proliferation. Thus IL-21 appears to terminate NK cell innate immunity by optimizing their function, triggering their elimination, and initiating T and B cell-mediated acquired immunity.

It is also clear that there is significant and important interaction between NK cells and dendritic cells. For example, cytokines such as IL-12 and IL-15 secreted by antigen-stimulated dendritic cells activate some NK cells. Thus the dendritic cells undertake their sentinel function and turn on NK cells, activating them and stimulating them to divide. The activated NK cells in turn secrete cytokines such as IFN γ that promote maturation of some DCs as well as promoting T cell priming.

30.4.5.1

CD95 Ligand Expression

CD95 ligand (CD95L) is normally expressed on cytotoxic T cells and NK cells. When it binds to CD95 on target cells, it triggers their apoptosis. CD95L has however also been detected on some leukemic T cells and NK cells, on colon adenocarcinoma cells, melanomas, and hepatocellular carcinomas. Since cytotoxic T cells may also express CD95, it is possible that cytotoxicity may work in reverse and that these CD95L⁺ tumor cells may kill T cells. At the same time, these cancer cells may downregulate their own CD95 so that they become resistant to cell-mediated cytotoxicity and cannot be killed by their own or a neighbor's CD95L. It is interesting to note that the anticancer drug doxorubicin enhances expression of both CD95 and CD95L on tumor cells and may permit these molecules to interact with T cells, thus killing themselves by apoptosis. Some tumor cells such as those in lung carcinomas may secrete decoy receptors for CD95L. These decoy receptors bind to CD95L and so block its binding to CD95. Thus tumor cells that downregulate CD95 while upregulating their decoy receptor expression may be resistant to cytotoxic cells.

30.5

SPECIES DIFFERENCES

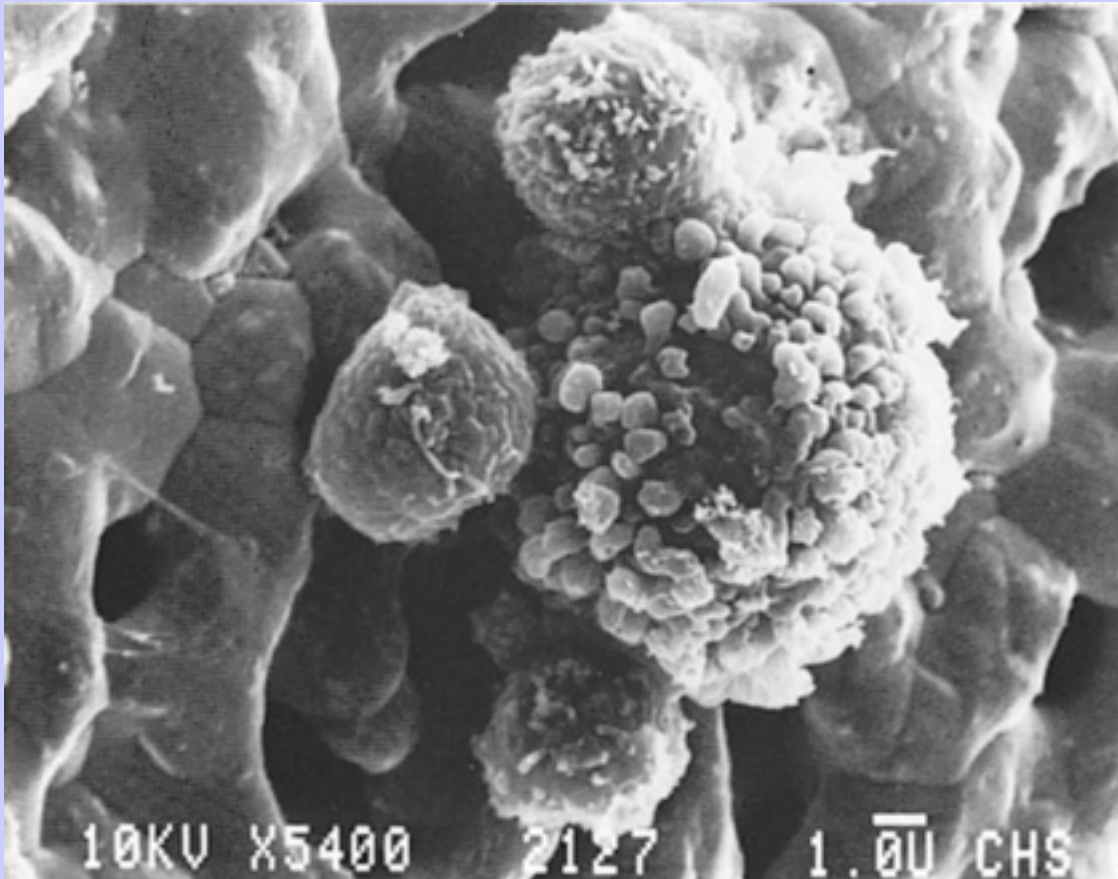
30.5.1

Horses

Equine NK cells are activated by recombinant human IL-2 and are active against human tumor cell targets. Horses have six transcribed *KLR* genes but no

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FIGURE 30-8 A scanning electron micrograph of natural killer cells from a pig (*small round cells*) attached to a target cell (*a human tumor cell*). Original magnification $\times 5400$. (From Yang WC, Schultz RD, Spano JS: *Vet Immunol Immunopathol* 14:345-356, 1987.)



transcribed *KIR* genes. This is surprising since it indicates that horse NK cell receptors resemble those of rodents rather than those of the other domestic mammals.

30.5.2

Cattle

NK cells constitute about 3.5% of blood lymphocytes in young calves and about 2% in older cattle. Bovine NK cells are $CD2^+$, $CD5^+$, $WC1^+$, MHC class II^+ , and asialo- GM_1^+ . (GM_1 is a ganglioside, which is a glyco-lipid molecule composed of a fatty ceramide residue buried in the lipid bilayer of a cell with at least three sugar groups projecting into the extracellular fluid. One of these is normally a sialic acid group. This is lacking in asialo- GM_1 .) Bovine NK cells also express CD94 and NKp46, as well as five members of the *KIR* family. Cattle have at least six *KIR* genes, some of whose products are inhibitory while others are activating. Cattle also have multiple *NKG2A*-related genes and at least one *Ly49* gene, suggesting that they possess more MHC-binding receptors than other species. Bovine NK cells are found in highest concentrations in the spleen and peripheral

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blood. In cattle, NK cells are large cells although they may not contain large intracytoplasmic granules. Bovine NK cells can attack human cancer cell targets and bovine cells infected with parainfluenza-3, bovine leukemia virus (BLV), and bovine herpesvirus type 1. They are activated by IL-2, IL-12, IL-15, IFN- α , and IFN- γ . Activation by IL-2 enables these cells to express CD25 and CD8 and lyse tumor cell lines. If activated by IL-12/15, their expression of granulysin, IFN- γ and perforin increases and they can kill both human tumor cells and bacillus Calmette-Guérin (BCG)-infected alveolar and monocyte-derived macrophages. Unlike other mammals in which the NKC occupies a single chromosomal region, the bovine NKC is divided between chromosomes 1 and 5.

30.5.3

Pigs

Pig NK cells are CD2⁺, CD8⁺, MHC class II⁺, and leukocyte function-associated antigen-1⁺. Pigs appear to have only a single KIR gene and only a single KLR gene. The latter may be inactive. Thus pigs appear to be unique in this respect and it raises the question as to just how pig NK cells recognize their targets. They are found in spleen and peripheral blood, but very few are found in lymph nodes or thymus ([Figure 30-8](#)). There is a debate about their morphology. Some investigators claim that they are large granular lymphocytes (LGLs), whereas others claim that they are small lymphocytes without obvious cytoplasmic granules. Porcine NK cells can lyse human cancer cells, as well as cells infected with transmissible gastroenteritis virus or pseudorabies virus.

30.5.4

Dogs

Canine NK cells can lyse distemper-infected target cells, as well as cancer cells from melanomas, osteosarcomas, and mammary carcinomas.

30.5.5

Cats

Feline NK cells are LGLs found in the blood and spleen. They are active against feline target cells infected with feline leukemia virus, herpesvirus, or vaccinia.

30.5.6

Chickens

Chicken NK cells are asialo-GM₁⁺ and may share determinants with T cells. Their morphology is unclear but they are probably LGLs. NK activity is found in the thymus, bursa, spleen, and intestinal epithelium. These cells can attack human cancer cells, lymphoid leukemia, leukemia virus, and Marek's disease virus-infected cells.

30.6

OTHER CELLULAR DEFENSES

30.6.1

Natural Killer T Cells

Natural killer T (NKT) cells are cells that share the properties of both NK and T cells. They are produced in the thymus, but their specificity is focused on just a few pathogen-associated molecular patterns. They circulate in the bloodstream, where they make up 0.5% to 1% of mononuclear cells in humans. They regulate diverse T cell responses ranging from anticancer responses to autoimmunity, allergy, and resistance to infections. NKT cells express invariant α/β TCRs in addition to NK cell receptors such as NK1.1 and members of the KLR family. Thus their antigen receptors resemble the receptors of innate immunity rather than those of acquired immunity.

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In mice, most NKT cells are either CD4⁺ or double negative. These NKT cells recognize glycolipid antigens from bacteria presented by the nonpolymorphic MHC class I molecule CD1d. Binding to glycolipids stimulates NKT cells, whereas binding to the KLR ligand inhibits their activity. NKT cells are activated by IL-15 early in an immune response but do not develop immunological memory. In Gram-negative infections, NKT cells are activated by IL-12 and IL-18 produced by dendritic cells in association with interactions between glycolipids bound to CD1d and the NKT receptor. However, they produce far more IL-4 and IFN- γ than do conventional T cells. During maturation these cells initially secrete IL-4 and IL-13 and thus have a Th2 phenotype. As they mature, however, they increase their secretion of IFN- γ and assume a Th1 phenotype. NKT cells therefore have major effects on other immune cells. They trigger chemokine and cytokine release, they enhance NK functions, and they promote dendritic cell maturation and B cell responses. They play a role in allergies, antitumor immunity, autoimmunity, and antimicrobial immunity (*especially that to Mycobacterium*). Thus they serve to link the T cell system with the innate NK cell system.

30.6.2 Natural Killer Dendritic Cells

There exists a population of cells, the NK dendritic cells, that possesses characteristics typical of both NK cells and dendritic cells. Thus they carry the NK cell marker NK1.1 as well as the dendritic cell marker CD11c. They are found in the spleen, liver, lymph nodes, and thymus of normal mice. They can lyse tumor cells but also present antigens to naïve, antigen-specific T cells. They produce copious amounts of IFN- γ on stimulation with CpG nucleotides. These cells may play a key role in linking innate and acquired immunity.

30.6.3 Conventional T Cells

It is occasionally possible to detect a cell-mediated response to tumor antigens by skin testing. Lymphocytes from some tumor-bearing animals may kill tumor cells cultured in vitro. Nevertheless, responses by T cells are probably significant only in controlling virus-induced tumors.

30.6.4 Macrophage-Mediated Immunity

In some experimental systems macrophages may have antitumor activities. This is especially true of M1 macrophages activated by exposure to IFN- γ . These M1 cells secrete cytotoxic molecules such as arginase and oxidants. Nonspecific activation of macrophages by BCG or *Propionibacterium acnes* results in enhanced production of IL-1 or TNF- α and subsequent activation of T helper cell and NK cell activity. IL-1 has a cytostatic effect on some tumors, and TNF- α may have potent antitumor activity. Unfortunately, malignant tumors may inhibit macrophage activation, and the macrophages of tumor-bearing animals may show defective mobilization and chemotaxis.

30.6.5 Antibody-Mediated Immunity

Antibodies to tumor cells are found in many tumor-bearing animals; for instance, about 50% of sera from dogs with lymphosarcomas contain precipitating antitumor antibodies. These antibodies may, together with complement, lyse free-floating tumor cells. Antibodies are not effective in destroying the cells in solid cancers.

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30.7 FAILURE OF IMMUNITY TO TUMOR CELLS

The fact that neoplasms are so readily induced and are so relatively common testifies to the inadequacies of the immunological protective mechanisms. Studies of tumor-bearing animals have indicated several mechanisms by which immune systems fail to reject tumors.

30.7.1 Immunosuppression

It is commonly observed that tumor-bearing animals are immunosuppressed. This suppression is most clearly seen in animals with lymphoid tumors: thus tumors of B cells tend to suppress antibody formation, whereas tumors of T cell origin suppress cell-mediated immune responses and NK cell activity. Immunosuppression in animals with chemically induced tumors appears to be due in part to production of immunosuppressive molecules, such as prostaglandins, from the tumor cells or from tumor-associated macrophages. The presence of actively growing tumor cells represents a severe protein drain on an animal. This protein loss may also be immunosuppressive.

Some tumor-derived molecules may redirect macrophage activities so that they promote tumor development. Thus tumor-derived IL-4, IL-6, IL-10, transforming growth factor β_1 , prostaglandin E_2 , and macrophage colony-stimulating factor can deactivate or suppress the activation of macrophages and suppress Th1 responses. Tumor cells can suppress macrophage cytokine production and circumvent macrophage cytotoxicity. Tumors may also evade T cell responses as a result of their failure to trigger inflammation and other innate responses.

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30.7.1.1 Regulatory Cells

Much of the immunosuppression seen in tumor-bearing individuals may be due to the activities of regulatory cell populations. These suppressor cells may be $CD8^+$ T_{reg} cells, IL-10-secreting Th2 cells, M2 macrophages, or even B cells. Enhanced suppressor cell activity can be detected in humans with osteogenic sarcomas, thymomas, myelomas, and Hodgkin's disease, and in many tumor-bearing animals. $FoxP3^+CD4^+$ T_{reg} cells may be increased in the blood and tumor-draining lymph nodes of dogs with cancer. Thus in normal dogs, $FoxP3^+$ cells constitute about 5% of blood T cells and 10% of lymph node T cells. In tumor-bearing dogs, however, they can constitute as many as 7.5% in blood and 17.1% in tumor-draining lymph nodes.

An example of the role of suppressor cells is seen in skin cancer induced by ultraviolet (UV) light in mice. Thus when UV radiation-induced skin cancer cells are transferred to the skin of normal allogeneic mice, they are rejected. When they are transferred to chronically UV-irradiated mice, they continue to grow. This is because immunosuppressive cells develop in the UV-irradiated skin before tumors appear. If adoptively transferred to normal mice, these suppressor cells prevent rejection of a subsequent tumor challenge. These suppressor cell populations are a mixture of T_{reg} cells and M2 macrophages.

Conventional cancer therapy may influence suppressor cell activity. Thus cyclophosphamide can inhibit suppressor-cell function, whereas immuno-stimulants such as *P. acnes* and BCG enhance suppressor cell activity in some patients, and this may account for the inconsistent results obtained with their use.

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30.7.1.2 Blocking Antibodies

Although tumor cells may be antigenic and stimulate a protective cell-mediated immune response, antibodies may have an opposite effect. Thus serum from tumor-bearing animals may cause the tumors of other animals to grow even faster—a phenomenon called enhancement. This serum may also inhibit T cell cytotoxicity. Many tumors release large quantities of cell surface antigen into the bloodstream, and this may bind to cytotoxic T cells, saturating their antigen receptors and so blocking their ability to bind to target cells. Alternatively, blocking antibodies may be produced. These are non-complement-activating, antitumor antibodies that can bind and mask tumor antigens and so protect the tumor cells from attack by cytotoxic T cells. In general, the presence or absence of these blocking factors correlates well with the state of progression of a tumor.

30.7.2 Tumor Cell Selection

Tumor cells don't usually become malignant in one step. Rather they gradually become malignant over a long period, going from benign to malignant in a process called tumor progression. The process occurs through a series of mutations that switch genes on and off. These mutations do not necessarily alter the immunogenicity of tumor cells or do so in small steps. Immunogenicity may not alter until the cells are irreversibly committed to malignancy. Thus there are two selection mechanisms by which tumor cells can evade the host's immune response and so enhance their own survival. One is “sneaking through,” the process by which malignant cells may not trigger an immune response until the tumor has reached a size at which it cannot be controlled by the host. Thus in experimental tumors, small numbers of tumor cells may grow after subcutaneous inoculation although large numbers may not. It may be that the tumor cells may not reach lymph nodes and trigger an immune response until the tumor burden is too large to be controlled. Even a very small tumor may contain an enormous number of cells. For example, a 10-mm tumor contains about 10^9 cells. The second mechanism reflects the fact that tumor cells that have mutated in such a way as to be antigenically different from the host will induce a strong immune response and be eliminated without leading to disease. Surviving tumor cells must therefore be selected for their lack of antigenicity and their inability to stimulate an immune response. To this extent, therefore, tumors that do develop have, by definition, already beaten the immune system.

30.8 TUMOR IMMUNOTHERAPY

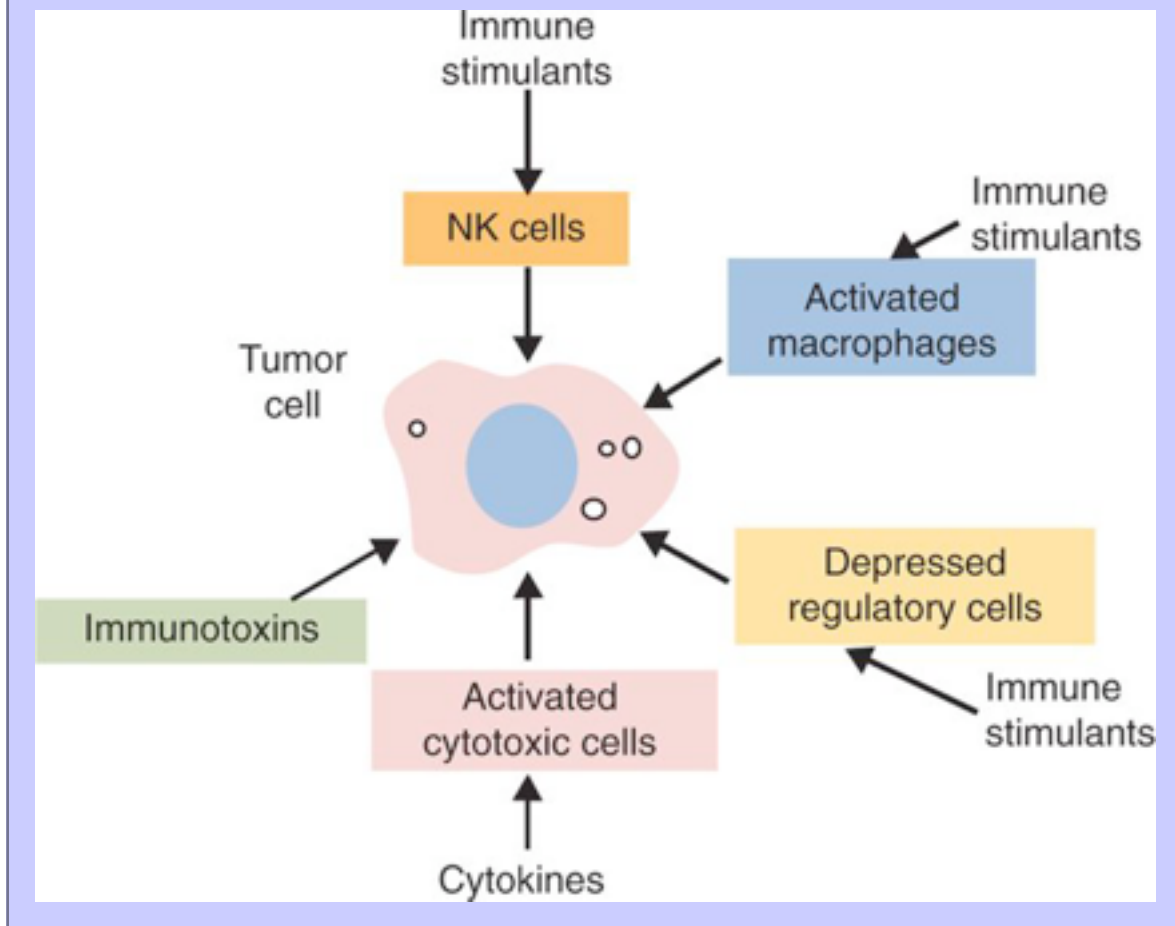
Immunotherapy may be either active or passive. In active immunotherapy the patient's own immune system is stimulated to respond to the tumor. In passive immunotherapy immune cells or their products are administered.

30.8.1 Active Immunotherapy

Three general approaches have been used in attempts to cure or modify tumor growth through immunotherapy ([Box 30-1](#)). The simplest is to stimulate the immune system nonspecifically ([Figure 30-9](#)). Any improvement in an animal's immune abilities will tend to

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FIGURE 30-9 Some of the ways in which the immune system can be stimulated to mount a protective response to tumors.



enhance its resistance to tumors, although a cure may be expected only if the tumor mass is small or is surgically excised. The most widely used immune stimulant is the attenuated strain of *Mycobacterium bovis* (BCG). This organism activates macrophages and stimulates cytokine release, thus promoting T cell activity. It may be given systemically or injected directly into the tumor mass. Most positive results from the use of BCG have come from studies on human patients with melanomas or bladder cancer. Direct injection of BCG into skin melanoma metastases may cause complete regression, not only of the injected lesion but also, occasionally, of uninjected skin metastases. However, visceral metastases usually remain unaffected. BCG enhances survival or remission length in some leukemias, and its direct intravesicular application in human bladder cancer gives complete or partial response rates of up to 70%. However, BCG can cause severe lesions at the site of injection and, occasionally, systemic hyper-sensitivity. Other immunostimulants that have been employed include *P. acnes*, levamisole, and various mixed bacterial vaccines.

30.8.1.1 Box 30-1 Some of the Approaches to Tumor Immunotherapy

30.8.1.1.1 Nonspecific Immune Stimulation

- Microbial products (bacille Calmette-Guérin, *Propionibacterium acnes*, yeast glucans, levamisole, etc.)
- Complex carbohydrates (acemannan)
- Cytokines (interferons, tumor necrosis factor, interleukin-2, interleukin-4)
- Lymphokine-activated killers (natural killer cells, T cells, tumor-infiltrating lymphocytes)

30.8.1.1.2 Passive Immunization

- Monoclonal antibodies against tumor antigens (alone or conjugated to toxins)

30.8.1.1.3 Active Immunization

- Chemically modified tumor cells
- DNA vaccination against related antigens in another species
- Vaccination against oncogenic viruses (feline leukemia, Marek's disease)

Many investigators have also studied the effects of vaccinating a patient with tumor cells or antigens. This approach has worked best in human melanoma patients, where several different antigen preparations are undergoing clinical trials. Because many tumors can evade the immune response, it is usual to treat the tumor cells in an attempt to enhance their antigenicity. Thus, X-irradiated, neuraminidase-, or glutaraldehyde-treated cells have been used in experimental tumor vaccines.

30.8.2 Passive Immunotherapy

30.8.2.1 Cytokine Therapy

Many attempts have been made to treat human cancer patients with isolated cytokines but with limited success. Interferons, for example, are only effective against certain selected tumors. Thus 70% to 90% of patients with hairy cell leukemia treated with IFN- α show complete or partial remission. The antitumor activities of TNF- α are synergistic with the interferons. On the other hand, administration of IL-2 to melanoma and renal cell cancer patients induces partial or complete remission in 15% to 20% of cases.

One major difficulty in cytokine therapy has been their toxicity. For example, when given at pharmacological doses, TNF- α produces clinical signs similar to those induced by endotoxin. IL-2 is also extremely toxic. In low doses it induces fever, chills, nausea, and weight gain as well as a capillary leak syndrome resulting in massive pulmonary edema. IL-2 also produces anemia, thrombocytopenia, and eosinophilia. Patients may also develop a severe, very itchy rash, neuropsychiatric changes, and endocrine abnormalities. Thus IL-2 is a hazardous protein with limited usefulness when used alone. Preliminary trials of IL-4 have shown similar

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toxic effects. Nevertheless it is important to note that local application of IL-2 may produce useful results. For example, relatively low doses of recombinant human IL-2, when injected locally into papillomas or carcinomas of the vulva in cattle, induced remissions in 83% of treated animals. Some complete regressions were observed. IL-2 therapy produced 63% complete remissions in cattle with ocular squamous cell carcinomas.

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30.8.2.2

LAK Cell Therapy

If NK cells or NKT cells are incubated in the presence of IL-2 for 4 days, they develop cytotoxic properties. Injection of these activated cells, called lymphokine-activated killers (LAKs), into mice with experimental lung tumors can lead to cancer remission. A combination of LAK cells and IL-2 has given encouraging results when administered to cancer patients.

LAK cells from blood include about 40% NK cells; the remainder are T cells. The activated NK cells are mainly CD3⁺, CD16⁺, and CD56⁺. They release LAK-1, a cytotoxic protein, as their effector molecule. IL-4 and IL-7 can also activate these cytotoxic cells. LAK cells have been induced in both cat and dog ([Figure 30-10](#)). Cell supernatants rich in IL-2 derived from concanavalin A-stimulated feline blood lymphocytes kill feline leukemia virus-transformed tumor cells. Recombinant human IL-2 stimulates the antitumor cytotoxic activity of canine blood lymphocytes.

In an attempt to obtain even better results in humans, tumors have been surgically removed from cancer-bearing patients and the lymphocytes within these tumors removed and cultured in the presence of IL-2 for 4 to 6 weeks so that their numbers grow significantly. These tumor-infiltrating lymphocytes recognize and infiltrate only the tumors from which they come. Given back together with IL-2 to the donor patients, they have produced remissions in about one third of patients. The most encouraging results have been obtained in patients with melanomas and those with colorectal and kidney cancer. Experimental injection of anti-CD152 (CTLA-4) into tumors in mice has also caused tumor regression. By blocking the suppressive receptor CD152, these antibodies permit T cell clones to expand and attack the tumor cells. Unfortunately, the chemokine environment within many tumors ensures that they are predominantly Th2 cells. The resulting inflammatory responses may therefore promote rather than inhibit tumor growth.

30.8.2.3

Antibody Therapy

Despite the risk of enhancement by antibodies, some successes have been achieved by the use of monoclonal antibodies for tumor therapy. Monoclonal antibodies can be used to destroy tumors, either when given alone or when complexed to highly cytotoxic drugs or potent radioisotopes, which they carry directly to the tumor cells. Thus a monoclonal antibody against canine T cells (CL/MAb231) has yielded encouraging results when used to manage lymphomas in dogs. It greatly increased life expectancy following two cycles of L-asparaginase/vincristine/cyclophosphamide/doxorubicin chemotherapy to bring the lymphoma into remission. The monoclonal antibody is given for 5 days, beginning 3 weeks after the conclusion of chemotherapy.

30.8.3

Successful Antitumor Vaccines

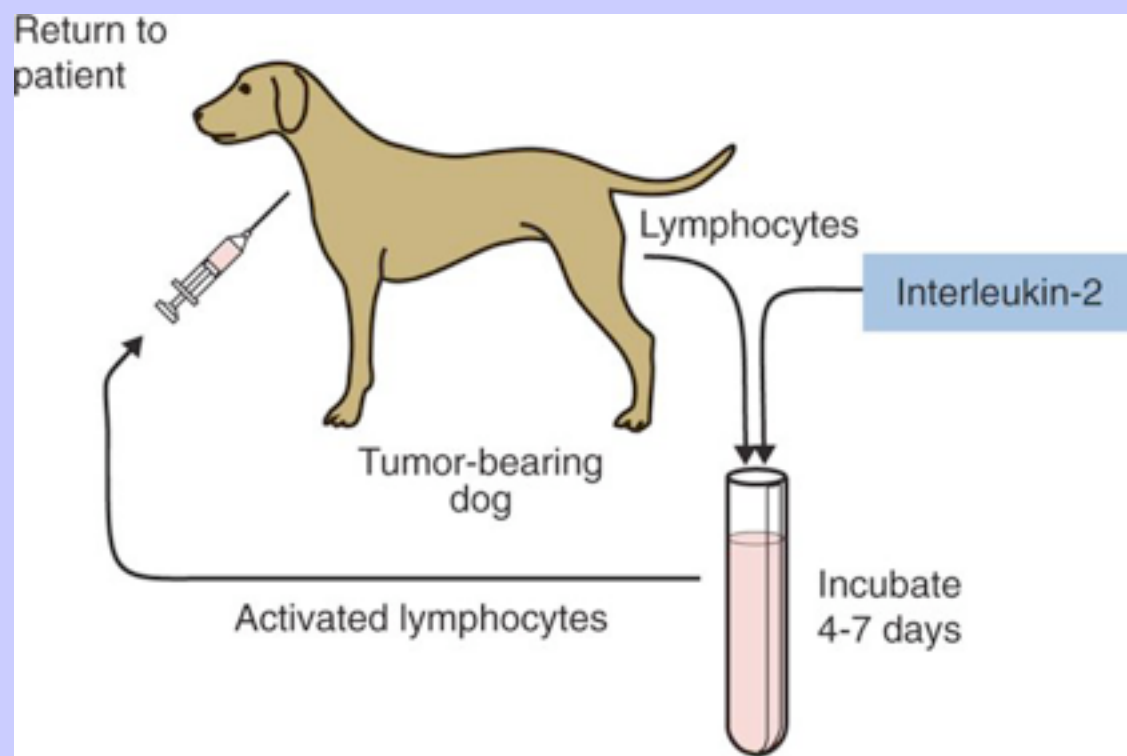
In contrast to the techniques described above, most of which have met with only limited success, there are established successful techniques for vaccination against tumor viruses. The most important of these are the vaccines against feline leukemia in cats. These vaccines usually contain high concentrations of the major viral antigens, and immunity is almost entirely directed against viral glycoproteins. Another important vaccine is that

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directed against Marek's disease, a T cell tumor of chickens caused by a herpesvirus. The immune response evoked by this vaccine has two components. First, humoral and cell-mediated responses act directly on the virus to reduce the quantity available to infect cells. Second, an immune response is provoked against virus-coded antigens on the surface of tumor cells. Both the antiviral and antitumor immune responses act synergistically to protect the birds.

A vaccine designed to enhance survival in recurrent canine melanoma has been approved by the US Department of Agriculture. This vaccine consists of an *Escherichia coli* expression plasmid engineered to express the human tyrosinase gene and administered transdermally by a needle-free device. The plasmid contains a cytomegalovirus promoter and a kanamycin resistance selection marker. Vaccinated dogs mount an immune response against the xenogeneic tyrosinase. Tyrosinase is a melanosomal glycoprotein that is essential for melanin synthesis. The immune

FIGURE 30-10 The production of lymphokine-activated killer cells by incubation of blood lymphocytes in the presence of interleukin-2 for 4 to 7 days.



response to the tyrosinase induces both antibodies and cytotoxic T cells against the melanoma cells, and this response will prevent tumor recurrence. Mean survival after receiving this vaccine was greater than 500 days, as opposed to 280-day survival in unvaccinated dogs.

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30.9 SOME SPECIFIC TUMORS

30.9.1 Transmissible Venereal Sarcoma

Transmissible venereal sarcoma is a neoplasm transmitted between dogs during copulation by transplantation of tumor cells. To colonize a new host, these cells must be capable of establishing themselves in allogeneic hosts. This is not always successful, and after an initial growth phase the tumor eventually regresses and is eliminated. Nevertheless, lethal metastases do occur in immunosuppressed dogs. When growing aggressively, these tumor cells fail to express β_2 -microglobulin and, as a result, MHC class I antigens are not assembled on the cell surface. Exposed dogs, whether or not they develop progressive neoplasms, develop antibodies to tumor cells, although the serum of dogs with regressive tumors is most effective in inhibiting tumor growth. In the regressive phase 30% to 40% of cells express MHC class I and II. Dogs whose tumors regress also develop cytotoxic T cells. If recipient dogs are immunosuppressed, the tendency to malignant growth is enhanced. These tumor cells appear to secrete a cytotoxic factor that kills B cells. Genetic analysis of these cells suggests that they are a clone of cells that originated in a wolf or east Asian breed of dog between 200 and 2300 years ago.

30.9.2 Papillomas

Warts are self-limiting neoplasms of epidermal cells induced by papillomaviruses. The wart virus invades epidermal cells in the basal cell layer, but these cells do not express viral antigen. Since no viral antigen is expressed in this area, where the blood supply is good, the cells are not attacked by lymphocytes. As the infected cells move away from the basal layer toward the skin surface, they also move away from blood vessels and the chances of immunological attack are minimized. Increasing amounts of virus are shed as the cells move toward the surface into a region devoid of antibodies or lymphocytes. Wart vaccines containing inactivated papillomavirus are available.

30.9.3 Equine Sarcoids

Equine sarcoids are locally aggressive fibroblastic neoplasms of horse skin associated with infection by bovine papillomavirus. Equine sarcoids are remarkably amenable to immunotherapy. If BCG vaccine is infiltrated into the tissues between the tumor and normal skin, the tumor regresses in about two thirds of cases. The rate of regression depends on the size of the tumor (surgical debulking is required to remove most of the tumor mass), and multiple treatments are usually necessary for a complete cure. Mycobacterial cell walls may also be used to eradicate this tumor. They possess the advantage of not rendering an animal tuberculin positive. Sarcoids are also responsive to other immunostimulants such as acemannan or killed *P. acnes*.

30.9.4 Ocular Squamous Cell Carcinoma

Ocular squamous cell carcinoma is a common and economically important tumor of cattle that responds to several forms of immunotherapy. One successful treatment involves inoculation of affected animals with a phenol-saline extract of allogeneic carcinomas. This suggests that these neoplasms possess characteristic tumor-associated antigens. Indeed sera from affected cattle can react with cancer cells (but not normal cells) obtained from the eyes of other cattle. It is also of interest to note that sera from some cattle with ocular squamous cell carcinoma also react with equine sarcoid and bovine papilloma cells, implying that all three may have a common cause.

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30.9.5 Swine Melanoma

The Sinclair melanoma-bearing swine is a line of animals that spontaneously develops melanomas. Most such tumors are benign and regress spontaneously. However, some are malignant and lethal. The tumor regression seen in most of these pigs is immunologically mediated. The tumors are invaded by macrophages, and at the same time the animals generate non-MHC-restricted cytotoxic T cells that are $CD4^-CD8^-$, γ/δ^+ . Recovering pigs may also generate antibodies against melanoma antigens.

30.10 LYMPHOID TUMORS

Acquired immunity requires that antigen-sensitive cells stimulated by exposure to antigen respond by division and differentiation. Much of the complexity of the immune system is due to the need for rigid control of this cellular response. A failure in this control system may result in uncontrolled lymphoid cell proliferation and the development of lymphoid tumors. Surveillance was originally proposed as a function of the immune system when it was observed that immunosuppressed animals and humans had an increased prevalence of tumors. However, analysis shows that an unusually high proportion of these tumors is of lymphoid origin. Therefore it is likely that at least some of the lymphoid tumors that develop in immuno-suppressed individuals result from a failure in the immunological control systems rather than from a failure of surveillance.

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Normal immune responses, whether antibody or cell mediated, involve a burst of rapid proliferation in lymphocytes. This burst of proliferation must be carefully controlled (see [Chapter 17](#)). Although uncontrolled lymphocyte function may induce autoimmunity, uncontrolled lymphocyte proliferation may result in the development of a lymphoma or lymphosarcoma. It is no accident that individuals with autoimmune disease are more likely than normal individuals to develop lymphoid cell tumors.

Several important viruses stimulate nonspecific lymphocyte proliferation. These include the maedi-visna virus, the Aleutian disease parvovirus, and the herpesvirus responsible for malignant catarrhal fever (MCF). MCF is a fatal lymphoproliferative disease of cattle and sheep characterized by lymphadenopathy with widespread tissue accumulations of lymphocytes. Lymphocytes from MCF-infected animals show prolonged growth in tissue culture.

Neoplastic transformation may occur in lymphoid cells of both branches of the immune system at almost any stage in their maturation process. Providing that the tumor cells have not dedifferentiated as a result of very rapid growth (as in acute lymphatic leukemia of calves), it is possible to identify the cells present in a lymphoid tumor by their surface antigens. For example, the presence of cell surface immunoglobulin is characteristic of B cells, whereas the presence of CD3 or CD2 is an identifying feature of T cells.

30.10.1 Bovine Lymphosarcoma

Bovine lymphosarcoma is one of the most common cancers of cattle. It occurs in two main forms: an enzootic form and a sporadic form. The enzootic form of the disease is caused by BLV, a delta retrovirus. BLV is transmitted by infected lymphocytes. Thus it can be spread by contaminated instruments, by vaccines containing blood, or by biting flies; or calves may be infected in utero. The primary target of the virus is the B cell. Early in infection the proportion of B cells in peripheral blood increases before there is a significant increase in the number of blood lymphocytes. Eventually some infected animals develop a persistent lymphocytosis (PL). Not all BLV-infected cattle develop PL, although 95% of cattle with this condition are infected with BLV. These lymphocytes may be enlarged, are $CD5^+$, express increased levels of immunoglobulin M (IgM), and show

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altered glycosylation. Cells in PL are not malignant and can occasionally return to the normal state. BLV becomes stably integrated into these B lymphocytes. Some T cells may also contain the BLV provirus. Susceptibility to tumor development differs among species. Sheep are very sensitive, cattle have intermediate sensitivity, and goats are the least sensitive. The virus is essential for neoplastic transformation but not for the continued growth of tumor cells.

The mechanism by which BLV leads to tumor development is unclear, since there is no rearrangement of any known oncogenes. A viral gene called Tax appears to initiate tumorigenesis. Tax is a transactivating protein that can turn on many different cellular genes and deregulates many different regulatory pathways rather than a single key pathway. Animals with advanced clinical bovine leukosis may be immunosuppressed as a result of the presence in their serum of a suppressor factor (Table 30-1). This suppression is reflected by reduced numbers of T cells and lowered serum IgM levels. Occasionally the neoplastic cells in bovine leukosis may be sufficiently differentiated to secrete immunoglobulin in a manner similar to that seen in myelomas. One to five percent of BLV-infected cattle develop a multicentric lymphosarcoma 1 to 8 years after infection. The cells in the sporadic form of bovine leukosis are predominantly T cells, but some originating from pre-B cells have also been identified.

30.10.2

Lymphomas in Other Species

In sheep, lymphomas are divided fairly evenly between T and B cells, and about 15% are unclassifiable (null cells). Some of these may be the result of BLV infection. A B cell lymphoma inherited as an autosomal recessive condition is recognized in swine. Horses carrying lymphosarcomas are commonly immunosuppressed. This usually involves T cell functions, but B cell function may also be impaired. A case of a horse with a lymphosarcoma with suppressor cell activity has been described. The animal presented with signs of immunodeficiency and was found to be deficient in IgM. The tumor cells grew in the presence of IL-2, possessed many T cell markers, and were noncytotoxic.

In dogs, leukemias may be classified on the basis of the cell type involved (lymphoid or myeloid) and on the basis of the clinical course and cytology (acute or chronic). Chronic lymphoid leukemia (CLL) is most frequently diagnosed. It is characterized by the presence of large numbers of mature lymphocytes in the blood. Animals may be asymptomatic, and the course of the disease is slow. About 70% of these cases involve T cells (CD3⁺), and most are LGLs. Of these LGLs, about 65% are α/β T cells and the remainder are γ/δ T cells. The non-LGL T cell CLL cases involve α/β T cells. Malignant B cells identified as CD21⁺ and CD79a⁺ account for about 30% of canine CLL cases. Chronic myeloid leukemias are extremely rare in the dog.

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Table 30-1 Immunosuppressive Effects of Lymphoid Tumors

Tumor	Cell Type	Evidence for Immunosuppression	Mechanisms
Feline leukemia	T cell	Lymphopenia Prolonged skin grafts Increased susceptibility to infection Lack of response to mitogens	Suppressive viral protein, p15E Suppressor cells
Marek's disease	T cell	Lack of response to mitogens Depressed cell-mediated cytotoxicity Depressed IgG production	Suppressor macrophages
Avian lymphoid leukosis	B cell	Increased susceptibility to infection	Suppressor lymphocytes
Bovine leukosis	B cell	Depressed serum IgM	Soluble suppressor factor
Myeloma	B cell	Increased susceptibility to infection	Soluble tumor-cell factor Negative feedback
Canine malignant lymphoma	B cell	Predisposition to infection associated with autoimmune disorders	Unknown
Equine lymphosarcoma	T cell	Increased susceptibility to infection	Tumor of suppressor cells

Acute leukemias, which are less common in dogs, may be of lymphoid (B cell) origin (20%), or myeloid origin (70%). The rest of these acute leukemias are difficult to classify and are considered undifferentiated. Many of these tumor cells, both myeloid and lymphoid, express CD34. The prognosis of these acute leukemias is usually very poor.

Lymphosarcomas account for 5% to 7% of canine malignancies. There is no evidence to suggest that these tumors are virus induced. They may be classified according to their apparent site of origin (such as multicentric, alimentary, or anterior mediastinal) or, alternatively, by their cell type (such as histiocytic, lymphocytic, lymphoblastic, or plasmacytic). The lymphocytic forms are usually of T cell origin. In many cases of canine lymphoma, affected dogs produce antibodies against crude tumor antigens. These antigens are not found on normal lymphoid cells.

Cutaneous T cell lymphomas (mycosis fungoides) are common in old dogs. The lesions consist of CD3⁺ cells. Eighty percent are CD8⁺, and the remainder are double negative. Most (70%) have γ/δ TCRs, especially if the tumor is confined to the epidermis.

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30.10.3

Avian Lymphoid Tumors

Marek's disease is a herpesvirus-induced tumor of T cell origin. Birds with this disease are usually immunosuppressed. Thus their antibody responses, rejection of allografts, and delayed hypersensitivity responses are all depressed. This depression results from several factors, including virus-induced lymphoid destruction and the development of suppressor macrophages. These macrophages restrict the replication of the tumor cells, but in doing so they suppress the resistance of birds to other infections. Lymphoid leukosis is a tumor of B cell origin. Affected birds normally have a depressed antibody response and reduced responses to mitogens. Nevertheless, some cases of this disease may present with hypergammaglobulinemia.

30.11

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31 CHAPTER 31 Autoimmunity: General Principles

31.1 KEY POINTS

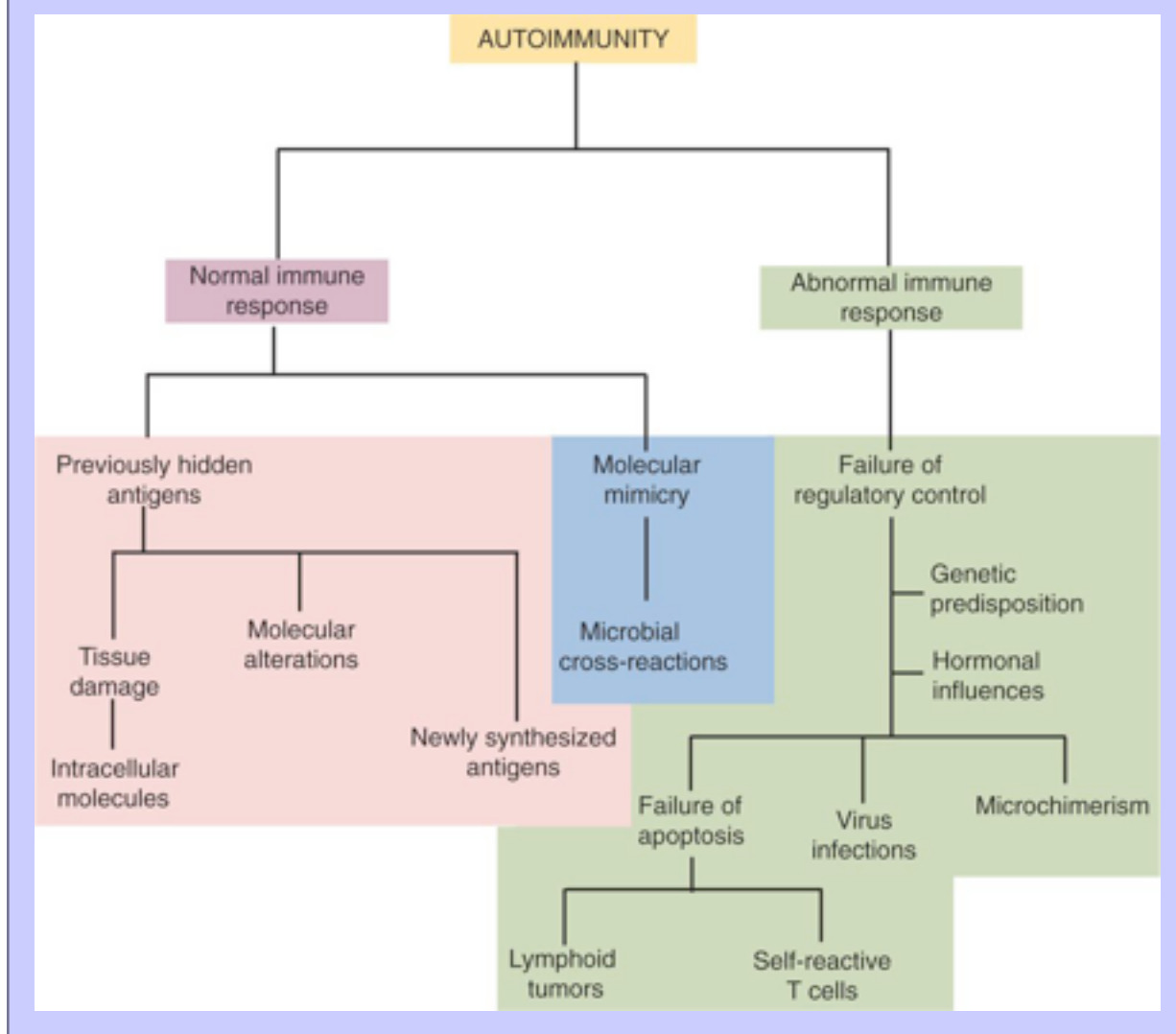
- Autoimmunity is an inescapable consequence of the way in which the acquired immune system has evolved.
- Not all autoimmunity is pathological. A normal autoimmune response may be mounted against antigens that develop late in life, against antigens hidden within cells, and against antigens that arise as a result of the development of new molecular configurations.
- Most autoimmune diseases result from a failure to ensure that tolerance is maintained against self-antigens. There may be a strong genetic predisposition to develop autoimmunity.
- Some autoimmune diseases are triggered by immune system stimuli such as virus infections, vaccination, and some drugs.
- The lesions that develop in autoimmune diseases correspond to the four major types of hypersensitivity.

An inescapable hazard associated with acquired immunity is the development of autoimmunity. By developing a defense system that can recognize any possible microbial antigenic determinant, vertebrates also developed the potential for self-destruction. The benefits of acquired immunity come at a high cost. The random generation of antigen-binding receptors ensures that lymphocytes are produced that can bind and respond to self-antigens. It has been estimated that 20% to 50% of T cell antigen receptors (TCRs) and B cell antigen receptors (BCRs) generated in this way will bind with high affinity to self-antigens. These self-reactive cells are rigorously suppressed so that only 3% to 8% of humans develop an autoimmune disease. However, why these individuals develop autoimmune diseases is still unclear. We know that many factors influence susceptibility to autoimmunity. These include sex and age, genetic background, and virus infections. We also know that the development of autoantibodies is a relatively common event that, by itself, does not inevitably lead to autoimmune disease. Indeed, some autoantibodies serve a physiological function.

Because we do not know precisely what causes autoimmune disease, this chapter reviews some of the many different predisposing factors that have been identified or proposed as well as the mechanisms by which autoimmunity causes tissue damage and disease.

As with other immune functions, both B and T cells can mediate autoimmunity. Thus in some autoimmune diseases the disease is mediated by autoantibodies alone. In others, the damage may be mediated by T cells alone or by some combination of autoantibodies and T cells.

FIGURE 31-1 Simplified scheme for the pathogenesis of autoimmune diseases.



31.2 INDUCTION OF AUTOIMMUNITY

Autoimmune diseases appear to develop spontaneously, and predisposing causes are rarely obvious. Nevertheless, they fall into two major categories. They can either result from a normal immune response to an unusual or abnormal antigen, or they can result from an abnormal immune response to a normal antigen ([Figure 31-1](#)). The second category is probably the most significant from the perspective of clinical disease. In these cases, the mechanisms that normally prevent the development of self-responsive T and B cells fail. Many different environmental factors and genes contribute to this failure, and the failure may not always be complete. Autoimmune diseases may result from an aberrant response to a single specific antigen, or, alternatively, they may be due to a general defect in the regulation of B or T cell functions.

31.3 NORMAL IMMUNE RESPONSES

Many autoimmune responses simply reflect a normal immune response to an antigen that has been previously hidden or, alternatively, are a result of cross-reactivity between an infectious agent and a normal body component.

31.3.1 Antigen Hidden in Cells or Tissues

Many autoimmune responses are triggered when nontolerant T cells meet previously hidden autoantigens. After all, T cells can only be made tolerant to autoantigens if the T cells are first exposed to these antigens. There are many autoantigens that do not induce tolerance because they are hidden within cells or tissues.

Although the control of the immune system requires that most self-reactive cells be eliminated, one should not assume that all autoimmune responses are bad or even cause disease. Indeed, some autoimmune responses have physiological functions. For example, red blood cells must be removed from the blood once they reach the end of their life span. This process is accomplished by autoantibodies. As red cells age, an anion transport protein called CD233 (or band 3 protein) is cleaved and a new epitope is exposed. This new epitope is recognized by immunoglobulin G (IgG) autoantibodies. These autoantibodies thus bind to aged red cells and trigger their phagocytosis by macrophages in the spleen. CD233 is also found on many other cell types, and it may be that its exposure in aged cells and their subsequent opsonization constitutes a major mechanism for their elimination.

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Many autoantigens are found in places where they never encounter circulating lymphocytes. For example, in the testes, new antigens may only appear at puberty—long after the T cell system has developed and become tolerant to autoantigens. Injury to the testes may permit proteins from damaged tissues to reach the bloodstream, encounter antigen-sensitive cells, and stimulate autoimmunity. Hidden antigens may also be found inside cells. For example, after a heart attack, autoantibodies may be produced against the mitochondria of cardiac muscle cells. In chronic hepatitis in dogs, animals develop antibodies to liver membrane proteins. In diseases in which widespread tissue damage occurs, such as trypanosomiasis or tuberculosis, autoantibodies to many different tissue antigens may be detected in serum.

31.3.2 Antigen Generated by Molecular Changes

The production of some autoantibodies may be triggered by the development of completely new epitopes on normal proteins. Two examples of autoantibodies generated in this way are the rheumatoid factors (RFs) and the immunoconglutinins (IKs, after the German spelling).

RFs are autoantibodies directed against other immunoglobulins. When an antibody binds to an antigen, the shape of the immunoglobulin molecule changes in such a way that new epitopes are exposed on its Fc region. These new epitopes may stimulate RF formation. RFs are produced in diseases in which large amounts of immune complexes are generated. These include the autoimmune disease of joints called rheumatoid arthritis and a disease called systemic lupus erythematosus (SLE), in which B cells respond to many different autoantigens.

IKs are autoantibodies directed against the complement components C2, C4, and especially C3. The epitopes that stimulate IK formation are exposed when these complement components are activated. The level of IKs in serum reflects the amount of complement activation; this, in turn, is a measure of the antigenic stimulation to which an animal is subjected. IK levels are thus nonspecific indicators of the prevalence of infectious disease within an animal population. Their physiological role is unclear, but they may enhance complement-mediated opsonization.

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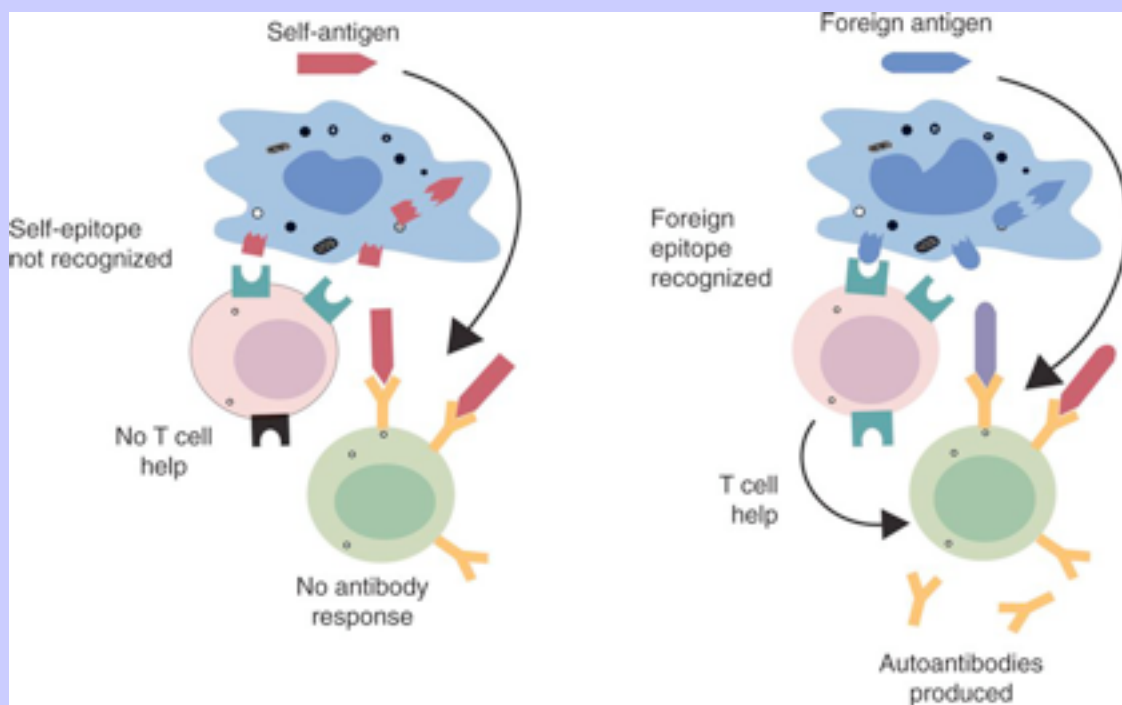
Minor structural changes in normal proteins may be generated artificially and used to induce autoantibodies. For example, chemically modified thyroglobulin can be used to induce autoantibodies against normal thyroglobulin.

31.3.3

Molecular Mimicry

Autoimmunity may result from molecular mimicry, a term used to describe the sharing of epitopes between an infectious agent or parasite and an autoantigen ([Figure 31-2](#)). Thus B cells may bind a foreign epitope that cross-reacts with an autoantigen. However, they

FIGURE 31-2 Cross-reactions with foreign antigens may be sufficient to trigger a T helper cell population that will promote an autoimmune response by B cells. Thus a helper effect triggered by a foreign antigen may inadvertently permit an autoimmune response to occur.



will only respond to this epitope if they receive T cell help. If Th cells recognize nearby microbial epitopes as foreign, they may trigger a response that permits the self-reactive B cells to make autoantibodies. Once a B cell response is triggered in this way, the infectious agent may be removed while the autoimmune response continues—a “hit-and-run” process.

Many examples of molecular mimicry are now recognized. For example, the parasite *Trypanosoma cruzi* contains antigens that cross-react with mammalian neurons and cardiac muscle. Individuals infected with *T. cruzi* make autoantibodies that can cause nervous system and heart disease. Molecular mimicry may also cause the heart lesions of rheumatic fever in children. Antibodies to the cell wall M protein of group A streptococci

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cross-react with cardiac myosin. Children infected with certain strains of group A streptococci produce antimyocardial antibodies and so develop heart disease. Other strains of streptococci may cause acute glomerulonephritis in children as a result of the production of antibodies cross-reacting with glomerular basement membranes. Other examples of molecular mimicry that may be significant include the Epstein-Barr virus DNA polymerase that cross-reacts with myelin basic protein and may be involved in the induction of multiple sclerosis, and poliovirus capsid protein VP2 that cross-reacts with the acetylcholine receptor and may induce myasthenia gravis.

The integrin CD11a/18 (LFA-1) shares an antigenic determinant with the outer surface protein of the agent of Lyme disease, *Borrelia burgdorferi*. Patients infected with this organism mount an initial immune response to the bacterium, which may then become an autoimmune response. In about 10% of patients with Lyme arthritis, antibiotics fail to resolve the disease suggesting that, once triggered, the autoimmune process can proceed in the absence of the bacterium.

Antibodies against microbial heat-shock proteins are found in the serum of humans and rats with rheumatoid arthritis, ankylosing spondylitis, and SLE. Injection of killed *Mycobacterium tuberculosis* in Freund's complete adjuvant can cause arthritis in rats, and T cells from these animals can transfer arthritis to normal syngeneic recipients. These T cells are responding to HSP 60, a mycobacterial heat-shock protein (see [Chapter 22](#)). Because heat-shock proteins are highly conserved and T cells from rheumatoid arthritis patients are also directed against HSP 60, it has been suggested that molecular mimicry between microbial and mammalian HSP 60 may be important in rheumatoid arthritis.

Ankylosing spondylitis is an autoimmune arthritis of humans that affects the sacroiliac joints, spine, and peripheral joints. Patients also develop acute anterior uveitis (inflammation of the iris and neighboring structures in the eye). More than 95% of caucasians with ankylosing spondylitis possess the major histocompatibility complex (MHC) class I allele human leukocyte antigen (HLA)-B27, whereas in the normal population the prevalence of this allele is less than 8%. It is believed that the disease results from molecular mimicry between the hypervariable region of HLA-B27 and antigens found in *Klebsiella pneumoniae* and related bacteria. *K. pneumoniae* is found more frequently than normal in the intestine of patients with active ankylosing spondylitis and uveitis, and patients with active disease have elevated levels of IgA against *Klebsiella* in their sera. Cloning of B27 into mice and subsequent infection of these animals with *K. pneumoniae* causes an acute spondylitis.

HLA-B27-like alleles have been cloned from bonobos, gorillas, rhesus monkeys, and cynomolgus monkeys, and HLA-B27-associated ankylosing spondylitis has been described in gorillas. In fact, up to 20% of wild gorillas may have spondylitis, and the disease has also been described in a gibbon, in baboons, and in rhesus macaques.

In porcine enzootic pneumonia caused by *Mycoplasma hyopneumoniae*, antibodies to the mycoplasma cross-react with pig lungs, and in contagious bovine pleuropneumonia there is cross-reactivity between *Mycoplasma mycoides* antigens and normal bovine lung. It is not known to what extent these autoantibodies contribute to the pathogenesis of these diseases. There is a clearer relationship between *Leptospira interrogans* infection and the development of periodic ophthalmia, the leading cause of blindness in horses (see [Chapter 32](#)).

Some microbial superantigens may also trigger autoimmunity. Thus the superantigen staphylococcal enterotoxin B activates the same T cells that react with myelin and induces an autoimmune encephalitis. It has been suggested that a bacterial superantigen may trigger rheumatoid arthritis since the T cells in affected joints are enriched in cells bearing certain TCR V domains. The only known agents that can alter V region gene expression in this way are superantigens.

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31.3.4 Receptor Editing

Both BCRs and TCRs are generated by random gene rearrangement. This process inevitably results in the generation of both nonfunctional and autoreactive antigen receptors. As lymphocytes develop, the primary lymphoid organs eliminate most of the cells that make ineffective or inappropriate receptors. Once a complete antigen receptor is formed, however, re-arrangement of the receptor gene segments continues. Thus if an immature B cell produces a receptor that binds to a self-antigen, the continuing development of the B cell is blocked while its light chain receptor chains continue to undergo recombination. This is an active process driven by the autoantigen. This replacement of one light chain by another leads to changes in receptor specificity and eventually makes the cells no longer autoreactive. Receptor editing to achieve tolerance occurs only in immature B cells. Mature B cells that bind autoantigen do not undergo receptor editing but are triggered to undergo apoptosis.

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The development of α/β T cells is similar to B cells in that it requires stepwise rearrangement and expression of the two antigen receptor loci. Editing occurs in double positive thymocytes prior to positive selection. The editing requires continued TCRA recombination, but its importance remains unclear. Presumably failures in TCR and BCR editing may provoke autoimmunity.

31.3.5 Alterations in Antigen Processing

In some cases autoimmunity seems to result from a normal immune response against an exogenous antigen that subsequently “spreads” to recognize self-antigens. Thus when an autoimmune response is initiated, the immune response is first directed against a single epitope on the inciting antigen. However, as the process continues, the T and B cell responses diversify and responses begin to be directed against additional epitopes. At first they will be other epitopes on the same protein. Eventually responses may spread to epitopes on other autoantigens. Epitope spreading has been demonstrated in many autoimmune diseases such as thyrotoxicosis and diabetes and may account for some of the difficulties encountered in controlling these diseases.

31.4 ABNORMAL IMMUNE RESPONSES

31.4.1 Failure of Regulatory Control

Although autoimmunity may be triggered in response to hidden epitopes, a sustained autoimmune response is necessary for disease to develop. This may result from a failure of the normal control mechanisms of the immune system and can be demonstrated simply by injecting mice with rat red blood cells. Following such an injection, mice not only make antibodies to the rat cells but also develop a self-limiting and transient autoimmune response to their own red blood cells. This autoimmune response is rapidly controlled by regulatory cells and lasts for only a few days. If, however, regulatory cell activity in these mice is impaired, as occurs in New Zealand Black (NZB) mice, for example, then these autoantibodies will persist to cause red blood cell destruction and anemia.

It is common to find autoimmune diseases associated with lymphoid tumors. For example, myasthenia gravis, an autoimmune disease involving the neuromuscular junction, is commonly associated with the presence of a thymic carcinoma. In humans, there is a fourfold increase in the incidence of rheumatoid arthritis in patients with malignant lymphoid tumors, and there is evidence for a similar association in other mammals. Since many lymphoid tumors result from a failure in immunological control mechanisms, a simultaneous failure in self-tolerance may also occur. Alternatively, some tumors may represent the development of a forbidden clone of

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cells producing autoantibodies. It is also possible that some lymphoid tumors may develop as a result of prolonged stimulation of the immune system by autoantigens.

Potentially harmful, self-reactive lymphocytes are normally destroyed in the thymus by apoptosis triggered through CD95 (fas) (see [Chapter 16](#)). Defects in CD95 or its ligand, CD154 (CD95L), cause autoimmunity by permitting abnormal T cells to survive and cause disease. This is well demonstrated in the *lpr* strain of mice. These animals have a mutation in their CD95 gene that alters the structure of its intracellular domain. A mutation (called *gld*) in fas ligand has a similar effect. Both *lpr* and *gld* mice develop multiple autoimmune lesions accompanied by lymphoproliferation. Some investigators have suggested that mutations in CD95 may contribute to the pathogenesis of lupus in other mammals. The AIRE (autoimmune regulator) gene permits multiple self-antigens to be expressed in thymic epithelial cells. T cells that respond to these self-antigens are destroyed. Thus humans with a defective AIRE gene develop a syndrome involving autoimmunity against multiple endocrine organs, the skin, and other tissues.

31.4.2

Virus-Induced Autoimmunity

Many autoimmune diseases appear to be triggered by virus infections. For example, mice infected with certain reoviruses develop an autoimmune polyendocrine disease characterized by diabetes mellitus and retarded growth. These reovirus-infected mice make autoantibodies against normal pituitary, pancreas, gastric mucosa, nuclei, glucagon, growth hormone, and insulin. Likewise, in NZB mice, persistent infection with a type C retrovirus leads to the production of autoantibodies against nucleic acids and red blood cells.

The situation with spontaneous disease is less clear. Many attempts have been made to isolate viruses from patients with autoimmune disease but with mixed results. For example, SLE of dogs and humans has been associated with either a type C retrovirus or paramyxovirus infection. Small quantities of the Epstein-Barr virus genome can be found in the salivary glands of humans with Sjögren's syndrome. Moreover, epidemiological evidence points to some form of a viral trigger for diseases such as multiple sclerosis, rheumatoid arthritis, and insulin-dependent diabetes mellitus in children.

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Just how viruses can induce autoimmunity is unclear. One possible mechanism described above is molecular mimicry. An equally plausible mechanism is called bystander activation. Thus viruses may induce an inflammatory response that results in the release of multiple cytokines. These cytokines may activate previously dormant T cells. As a result the T cells may attack autoantigens that they previously ignored. Evidence suggests that Coxsackie virus-induced diabetes is mediated in large part through bystander activation ([Figure 31-3](#)).

31.4.3

Microchimerism

During pregnancy mothers and their fetuses may exchange cells. As a result, fetal cells may persist in a mother's body for many years after pregnancy. Conversely, a mother's cells may survive for many years in her offspring. These cells are accepted by a tolerant immune system. These persistent cells may be the cause of some autoimmune diseases. This is especially true in humans where autoimmune diseases are much more common in women than in men. The process is called fetal microchimerism. Thus in many women with the autoimmune disease scleroderma, it is possible to find fetal T, B, and natural killer cells as well as fetal monocytes in their blood. It is suggested that scleroderma is a form of graft-versus-host disease in these patients. Fetal cells have also been identified in humans with autoimmune thyroiditis. Transfer of cells from mother to fetus may also cause autoimmunity. Thus small numbers of maternal cells can be detected in the blood of almost all boys with the autoimmune disease dermatomyositis. In all of these cases the number of foreign cells in an individual is clearly insufficient to be the sole cause of the autoimmune disease, so additional factors must be involved.

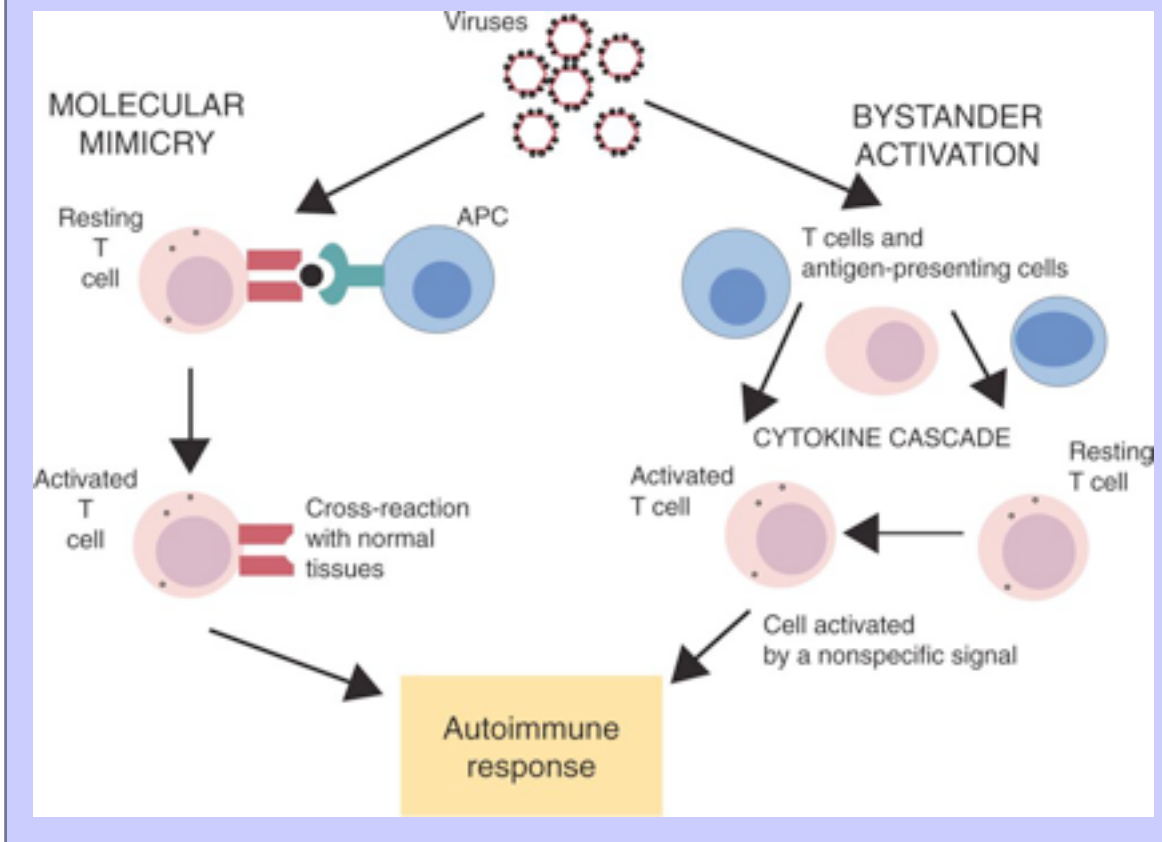
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31.5 PREDISPOSING FACTORS

31.5.1 Genetic Predisposition

Although viruses or other infectious agents may trigger autoimmune responses, it is clear that not all infected individuals develop autoimmune disease. This is because genetic factors are key determinants of disease susceptibility. Genetic analysis of mice has led to the identification of at least 25 genes that contribute to autoimmunity if deleted or overexpressed. These include genes that code for cytokines, cytokine receptors, co-stimulators, molecules that regulate apoptosis, molecules that regulate antigen clearance, and members of cytokine or antigen-signaling cascades. Some diseases result from a defect in a single gene,

FIGURE 31-3 Viruses may trigger autoimmune responses either by molecular mimicry or by bystander activation.



such as the *aire*, *lpr*, or *gld* mutations. Their gene products play a key role in the destruction of self-reactive T cells. In their absence, excessive T cell proliferation and autoimmunity result. Others result from inherited complement deficiencies. More commonly, the role of genes is complex. Thus genes influence the severity of disease and no specific gene is necessary or sufficient for disease expression. Even if an animal has a complete set of susceptibility alleles at multiple loci, presence of overt disease may depend on the genetic background of the animal. (This is called incomplete penetrance.) This genetic complexity probably also contributes to differences in disease presentation since these may be determined by different sets of contributing genes. Genetic

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analysis is also complicated because different susceptibility genes may or may not interact with each other. The vulnerability of a target organ to autoimmune damage may also be inherited.

The most important genes that influence naturally occurring autoimmune diseases are those in the MHC. MHC molecules regulate the presentation of processed epitopes. In theory, therefore, they determine resistance or susceptibility to many diseases. In practice, there is strong selection against genes that predispose to susceptibility to infectious agents, so that MHC genes have been selected for a strong response to most common infectious pathogens. In contrast, autoimmune diseases in old, postreproductive animals do not offer a selective disadvantage and MHC-linked predispositions can be identified. Studies of human populations have shown that almost all autoimmune diseases are linked to certain MHC alleles. Presumably an essential prerequisite for any autoimmune disease is that the autoantigen is appropriately processed and presented on an MHC molecule. Thus the structure of the MHC antigen-binding groove determines if a specific autoantigen will trigger an immune response. Some MHC alleles protect against autoimmunity, and any predisposition to autoimmunity may be the result of the net effect of both enhancing and protective genes.

Instead of being closely linked to a single MHC gene, some autoimmune diseases are associated with combinations of MHC molecules. For example, in humans the combination of HLA-A1, B8, and DR3 is associated with increased risk of diabetes, myasthenia gravis, and SLE.

Many domestic dogs, especially those from rare breeds, are significantly inbred and show restricted MHC polymorphism. This can increase autoimmune disease susceptibility. In the dog there are several recognized associations between autoimmunity and MHC alleles. Thus diabetes mellitus is associated with dog leukocyte antigen (DLA)-A3, A7, and A10 and DLA-B4; antinuclear antibodies are associated with DLA-12; SLE is associated with DLA-A7; and autoimmune polyarthritis is associated with certain C4 alleles.

31.5.2

Breed Predispositions

The three major classes of immunologically mediated disease (autoimmunity, immunodeficiency, and atopy) tend to be encountered in some dog breeds more commonly than in others. Thus Old English Sheepdogs are unusually prone to develop autoimmune blood diseases. Certain autoimmune diseases, such as poly-arteritis nodosa and hypothyroidism, have familial associations (see [Chapter 32](#), [Table 32-1](#)).

Breeds are, of course, an artificial phenomenon. They have been developed as a result of aggressive phenotypic selection, in many cases resulting in inbreeding and a lack of genetic diversity. This has had two effects. First, it has permitted deleterious autosomal recessive genes to be expressed, as is seen in the increased prevalence of immunodeficiency syndromes and other immunological disorders. Second, it has resulted in a loss of MHC polymorphism. For example, DRB1*04 is found in a majority of Boxers, DRB1*2401 may be restricted to Akitas, DRB1*01 predominates in West Highland White Terriers, DQA*0203 is restricted to Dobermans, there is a high incidence of DQA*0102 in Irish Wolfhounds and Chows, and DRB1*0101 is common in Irish Setters. These limited haplotypes ensure that the dogs in these breeds will respond to an unusually narrow range of antigens, thus reducing their resistance to infectious agents. Such dogs will also be more susceptible to autoimmune diseases because of restricted range of immune responses they can mount. The increasing incidence of immunological diseases seen in dogs is largely attributable to careless breeding practices.

Inbred lines of other species have been produced that are associated with spontaneous development of autoimmune disease. For example, chickens of the OS strain develop an autoimmune thyroiditis. Inbred NZB mice spontaneously develop a syndrome that bears a striking resemblance to SLE (see [Chapter 33](#)). These mice develop immune complex glomerulonephritis. They become hypergammaglobulinemic and

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hypocomplementemic, and they develop an autoimmune hemolytic anemia. Some also develop lymphoid tumors. NZB mice produce autoantibodies against nuclear antigens, red blood cells, and T cells, and their B cells are polyclonally activated. New Zealand White (NZW) mice are phenotypically normal, but the F1 cross between NZW and NZB mice has an even more severe lupus-like syndrome. In these animals, kidney disease is severe and is associated with high titers of antibodies to nucleic acids. Studies on the inheritance of these traits in mice suggest that they are controlled by a small number of unlinked major genes and a large number of minor genes.

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31.6 MECHANISMS OF TISSUE DAMAGE IN AUTOIMMUNITY

Autoimmune disease results when tissues are damaged by autoreactive T cells or antibodies. This damage is a result of hypersensitivity reactions. However, it should be pointed out that multiple mechanisms may be involved and that these may vary with time.

31.6.1 Type I Hypersensitivity

Milk allergy in cattle is an autoimmune disease in which milk (α casein), normally found only in the udder, gains access to the general circulation and so stimulates an immune response. This happens when milking is delayed and intramammary pressure forces milk proteins into the circulation. For some reason the immune response stimulated by a casein is mediated through Th2 cells, and IgE autoantibodies are produced. As a result, affected cows may develop acute anaphylaxis (see [Chapter 25](#)). A similar condition is seen occasionally in other domestic mammals such as the mare. Although antibodies in milk proteins are commonly found in human serum after rapid weaning, type I hypersensitivity is not a usual sequel.

31.6.2 Type II Hypersensitivity

Autoantibodies against cell surface antigens may cause cell lysis with the assistance of complement or cytotoxic cells. If autoantibodies are directed against red blood cells, autoimmune hemolytic anemia may result; if directed against platelets, thrombocytopenia will occur; and if against thyroid cells, thyroiditis will result. In one form of this process in humans, auto-antibodies against thyroid-stimulating hormone (TSH) receptors on thyroid cells stimulate thyroid activity rather than its destruction. Cell surface receptors are common targets of autoimmune attack. In addition to the TSH receptor, autoantibodies attack the acetylcholine receptor in myasthenia gravis, and the insulin receptor in some forms of diabetes. Autoantibodies to β -adrenoceptors (see [Chapter 25](#)) have been detected in some patients with asthma. By blocking β -receptors, these antibodies make the airways highly irritable and affected individuals are prone to severe asthma.

31.6.3 Type III Hypersensitivity

Autoantibodies form immune complexes with auto-antigens, and these complexes may cause inflammation. This is most significant in SLE, a disease in which many different autoantibodies are produced. Immune complexes deposited in glomeruli provoke a membranoproliferative glomerulonephritis (see [Chapter 27](#)). Similarly, in rheumatoid arthritis, immune complexes are deposited in joints and contribute to the local inflammatory response.

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31.6.4

Type IV Hypersensitivity

Many autoimmune disease lesions are infiltrated with mononuclear cells, and Th1 responses probably contribute to the pathogenesis of disease of this type. Cytotoxic T cells cause demyelination in experimental allergic encephalitis and human multiple sclerosis. Insulin-dependent diabetes mellitus may be due to a cell-mediated response because the diseased pancreatic islets may be infiltrated by lymphocytes, and lymphocytes from diabetics may be cytotoxic for pancreatic islet cells in vitro.

Although cytotoxic T cells can kill cells directly, cytokines from these cells may also cause tissue damage. Examples of this include interleukin-1, which stimulates nitric oxide production; the nitric oxide in turn kills cells. Likewise, tumor necrosis factor- α released by these cells is proinflammatory and upregulates cell adhesion molecules, including selectins, and so facilitates immigration of neutrophils into the lesions.

31.7

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³² CHAPTER 32 Organ-Specific Autoimmune Diseases

^{32.1} KEY POINTS

- Domestic mammals suffer from a diverse array of autoimmune diseases. There is no reason to suppose that any organ or tissue is not a potential victim of autoimmune attack.
- The most common autoimmune diseases in domestic mammals involve the endocrine system, the skin, and blood cells.
- Treatment of autoimmune diseases usually involves suppression of the destructive inflammatory lesion with corticosteroids. This may be supplemented by the use of immunosuppressive drugs.

This chapter considers those autoimmune diseases that mainly affect a single organ or tissue. These diseases presumably result from an abnormal response to a small number of self-antigens and do not necessarily reflect significant loss of control of the immune system as a whole. It is likely that all organs of the body are potentially susceptible to this form of immunological attack.

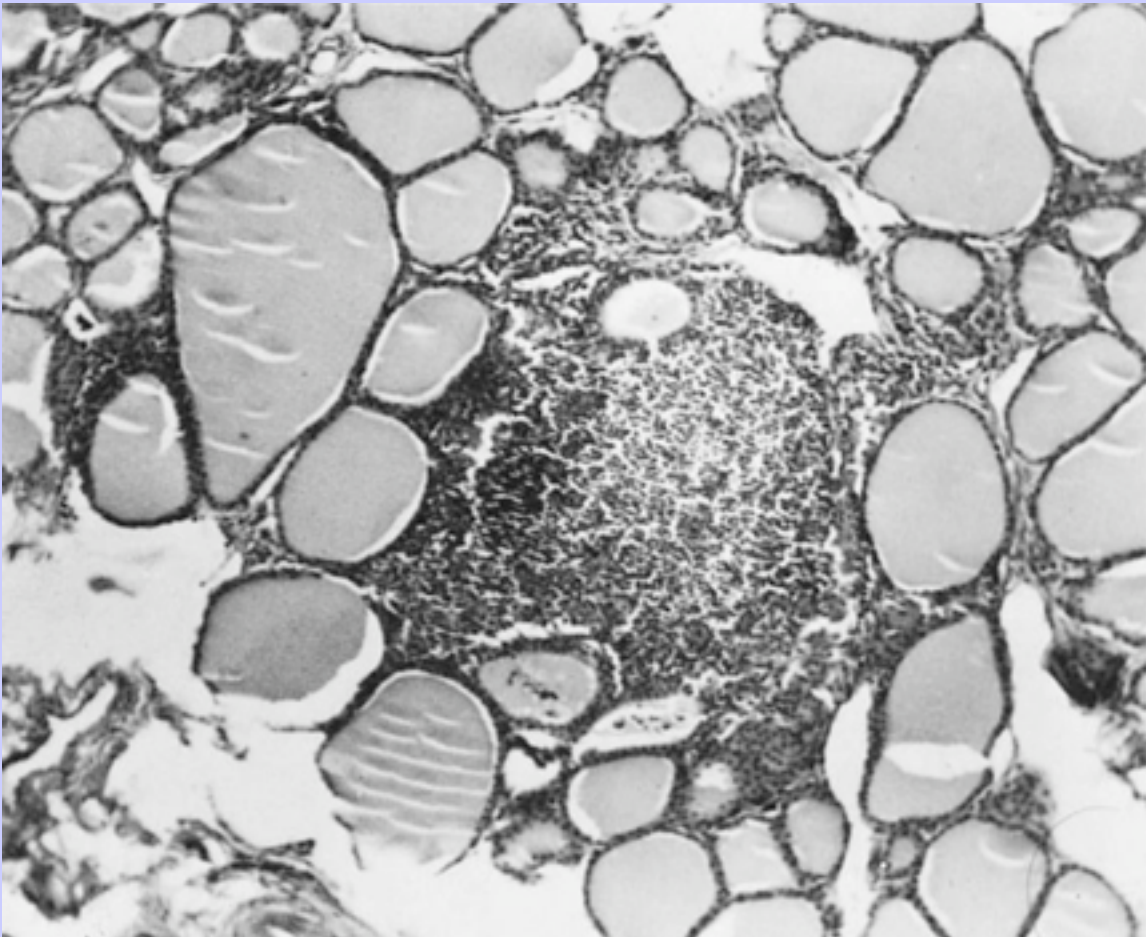
^{32.2} AUTOIMMUNE ENDOCRINE DISEASE

Although domestic animals develop autoimmune endocrine diseases, they differ from those in humans insofar as they tend to present as single disorders, rather than involving multiple endocrine glands. Occasionally a dog may experience two or more autoimmune endocrine disorders simultaneously (autoimmune polyglandular syndrome), but this is very uncommon.

Table 32-1 Breed Susceptibility to Major Autoimmune Diseases

	Thyroiditis	IDDM	Pancreatitis	Meningitis	VKH	IMHA	AITP	MG
Great Danes	+							
Borzoi	+							
Doberman Pinschers	+							
Golden Retrievers	+				+			
Dachshunds	+							
Cocker Spaniels	+					+	+	
Miniature Schnauzers	+							
Irish Setters	+				+	+		
Beagles	+							
Old English Sheepdogs	+					+	+	
Samoyeds		+			+	+		
German Shepherds			+	+				
Rough-Coated Collies			+					
Boxers				+				
Bernese Mountain Dogs				+				
Siberian Huskies					+			
Saint Bernards					+			
Australian Sheepdogs					+			
Shetland Sheepdogs					+			
Akitas					+		+	
Miniature Dachshunds						+		
Scottish Terriers						+	+	
Vizslas						+		
Poodles							+	
Short-Haired Pointers							+	
Chihuahuas							+	

FIGURE 32-1 A lymphocytic nodule in the thyroid of a dog suffering from autoimmune thyroiditis. Original magnification $\times 100$. (From a specimen provided by Dr. B.N. Wilkie.)



32.2.1 Lymphocytic Thyroiditis

Dogs, humans, and chickens suffer from autoimmune thyroiditis. It results from the production of autoantibodies against thyroglobulin or thyroid peroxidase. These antibodies may also react with triiodothyronine (T_3) or thyroxine (T_4). Affected dogs may also show a delayed skin reaction to intradermally injected thyroid extract, suggesting that cell-mediated mechanisms also participate in the disease. Several dog breeds are predisposed to the disease ([Table 32-1](#)), and relatives of affected animals may have antithyroid antibodies although clinically normal. A familial form of hypothyroidism has been demonstrated in beagles and Great Danes. Dogs from high-risk breeds such as Dobermans tend to develop the disease when young, whereas dogs from low-risk breeds tend to develop it when older. Unfortunately, by the time the disease is diagnosed the dog may already have been bred several times. Affected thyroids are infiltrated with plasma cells and lymphocytes, and germinal center formation

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may occur ([Figure 32-1](#)). The invading lymphocytes probably cause epithelial cell destruction through antibody-dependent cell-mediated cytotoxicity (ADCC) and T cell cytotoxicity.

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In dogs clinical signs appear when about 75% of the thyroid is destroyed. These signs are those of hypothyroidism; that is, the animals are fat and inactive and show patchy hair loss. The most common problems are a dry, dull, coarse coat; scaling; hypotrichosis; slow hair regrowth; hyperpigmentation; myxedema; and pyoderma. Other signs include myopathy, hyperlipidemia, hypothermia, anestrus, galactorrhea, diarrhea or constipation, and polyneuropathy. Tests of thyroid function such as a radioimmunoassay for plasma T_4 or T_3 confirm only the existence of hypo-thyroidism. A thyroid-stimulating hormone (TSH) response test is more useful because it can confirm the inability of the affected thyroid to respond to TSH. (Plasma T_4 levels are measured before and after injection of TSH.) To confirm autoimmune thyroiditis, a biopsy must show the characteristic lymphocytic infiltration. Antithyroid antibodies must be detected in serum using enzyme-linked immunosorbent assay, immunoblots, or an indirect fluorescent antibody test (see [Chapter 38](#)). There is little correlation between antithyroid antibody titers and disease severity. Management of autoimmune thyroiditis involves replacement therapy with sodium levothyroxine (synthetic T_4). Improvement should be seen within 4 to 6 weeks. There is no cure for this disease, and the success of treatment depends on effective replacement therapy.

An autoimmune thyroiditis also occurs in the OS (obese) strain of white Leghorn chickens. The thyroid tissue of these birds is heavily infiltrated by lymphocytes and plasma cells. Autoantibodies are directed against thyroglobulin, and affected birds are hypothyroid. These birds also make antibodies against their adrenal gland, exocrine pancreas, and proventricular cells. Neonatal thymectomy prevents the development of lesions.

32.2.2 Hyperthyroidism

Hyperthyroidism is a disease of old cats. Autoantibodies to thyroid peroxidase have been demonstrated in almost one third of cases of feline hyperthyroidism, and about 10% of these animals also have antinuclear antibodies. Lymphocytic infiltration is observed in about one third of cases, and it is possible that these cases may be immunologically mediated.

32.2.3 Lymphocytic Parathyroiditis

Dogs and cats develop autoimmune hypoparathyroidism. Affected animals usually have a history of neurological or neuromuscular disease, especially seizures. On investigation, affected animals are profoundly hypocalcemic and serum parathormone levels are severely reduced. Normal parathyroid tissue is replaced by a massive infiltration of lymphocytes and a few plasma cells. Once hypocalcemic tetany is controlled, these animals may be treated by oral vitamin D and calcium administration. It would be logical to administer immunosuppressive therapy.

32.2.4 Insulin-Dependent Diabetes Mellitus

In humans, insulin-dependent diabetes mellitus (IDDM) is an autoimmune disease mediated by autoantibodies against an islet cell enzyme called glutamic acid decarboxylase. At least some cases of spontaneous IDDM in dogs may also be immunologically mediated. The canine disease is associated with pancreatic islet atrophy and a loss of β cells. In some cases, the islets are infiltrated by lymphocytes. Experimentally, circulating mononuclear cells from diabetic dogs have caused cultured mouse islet cells to release insulin. If these islet cells were stimulated to release insulin by exposure to glucose, then the mononuclear cells suppressed insulin release.

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Serum from IDDM dogs lysed these islet cells in the presence of complement (Figure 32-2). When dog serum was tested for antibodies against cultured β cells by immunofluorescence, 9 of 23 diabetic dogs showed strongly positive reactions and an additional 3 showed a weak reaction. Only 1 of 15 normal dogs gave a positive response. Thus cytotoxic cells or antibodies or both may be responsible for β cell destruction in dogs. Therefore if autoimmune diabetes is diagnosed, treatment should include immunosuppressive therapy including prednisolone, cyclophosphamide, or azathioprine. A familial predisposition to IDDM has been observed in Samoyeds.

Diabetes mellitus is rare in cattle. Affected animals have atrophied and reduced numbers of pancreatic islets with partial or complete loss of β cells. Lymphocytes commonly infiltrate the remaining islets.

32.2.5 Atrophic Lymphocytic Pancreatitis

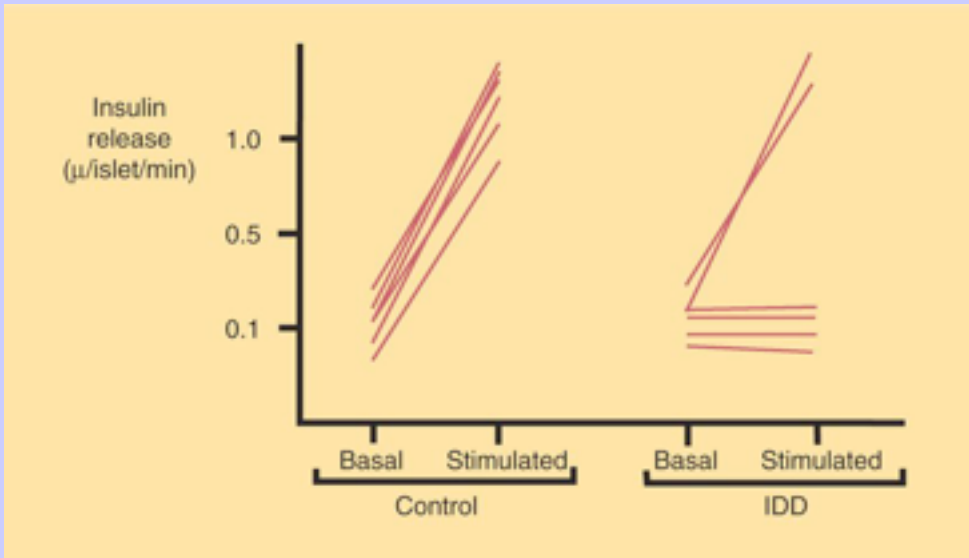
The most common cause of an exocrine pancreatic deficiency in dogs is an atrophic condition associated with lymphocyte infiltration. It is seen predominantly in German Shepherds and Rough-Coated Collies. The infiltrating lymphocytes are primarily $CD4^{+}$ and $CD8^{+}$ T cells. The $CD8^{+}$ cells are associated with areas of pancreatic necrosis. Some of these dogs have low levels of antibodies against pancreatic acinar cells, so this may be an autoimmune disease.

32.2.6 Autoimmune Adrenalitis

Dogs may suffer from lymphocyte-mediated destruction of the adrenal cortex. Affected animals present

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FIGURE 32-2 Insulin release from islets incubated in vitro in the presence of six individual control or insulin-dependent diabetic dog sera, plus complement. Four of the six diabetic dog sera inhibited insulin release from cultured islets. (From Sai P, Debray-Sachs M, Jondet A, et al: *Diabetes* 33:135-140, 1984.)



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with depression, weak pulse, bradycardia, abdominal pain, vomiting, diarrhea, dehydration, and hypothermia. As a result of excessive sodium and chloride loss, animals develop hypovolemia and acidosis leading to circulatory shock, hyperkalemia, and cardiac dysrhythmias. Blood corticosteroid levels are low in these animals. This disease has been observed in association with hypothyroidism.

32.3 AUTOIMMUNE NEUROLOGICAL DISEASE

An autoimmune brain disease known as experimental allergic encephalomyelitis may be produced by immunizing animals with brain tissue emulsified in Freund's complete adjuvant. After a few weeks, dogs or cats develop erratic focal encephalitis and myelitis, possibly with paralysis. The brain lesions consist of focal vasculitis, mononuclear cell infiltration, perivascular demyelination, and axon damage. Antibodies to brain tissue can be detected in the serum of these animals, although the lesion itself is a result of a cell-mediated response.

Similar encephalitis used to occur following administration of rabies vaccines containing brain tissue to humans. For this reason, the use of adult brain tissue was stopped and suckling mouse brain tissue taken before myelination is used in the production of rabies vaccines. Postdistemper demyelinating leukoencephalopathy may also be of autoimmune origin, although the production of antimyelin antibodies appears to be a common response to central nervous tissue damage, regardless of its cause.

32.3.1 Equine Polyneuritis

Equine polyneuritis (neuritis of the cauda equina) is an uncommon disease of horses affecting the sacral and coccygeal nerves. Affected horses show hyper-esthesia followed by progressive paralysis of the tail, rectum, and bladder and localized anesthesia in the same region. The disease may also be associated with facial and trigeminal paralysis. Although sacral and lumbar involvement is usually bilateral, the cranial nerve involvement is often unilateral. A chronic granulomatous inflammation develops in the region of the extradural nerve roots. Affected nerves are thickened and discolored. They show a loss of myelinated axons; infiltration by macrophages, lymphocytes, giant cells, and plasma cells; and deposition of fibrous material in the perineurium. In severe cases the nerve trunks may be almost totally destroyed. Affected horses have circulating antibodies to a peripheral myelin protein called P2, which can induce experimental allergic neuritis in rodents (see later). Although equine polyneuritis may be an autoimmune disease, equine adenovirus 1 has been isolated from its lesions, so that the cause is complex. Because of the severe nerve damage, immunosuppressive or antiinflammatory therapy is rarely successful.

32.3.2 Canine Polyneuritis

Canine polyneuritis or coonhound paralysis affects dogs following a bite or scratch from a raccoon. It presents as an ascending symmetrical flaccid paralysis with mild sensory impairment. The bitten limb is usually affected first but the disease is progressive and will worsen for 10 to 12 days following the bite. In

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FIGURE 32-3 A section of rat sciatic nerve showing a mononuclear cell infiltration. This is the lesion of experimental allergic neuritis produced by inoculation of rat sciatic nerve in Freund's complete adjuvant. Original magnification $\times 400$. (Courtesy Dr. B.N. Wilkie.)

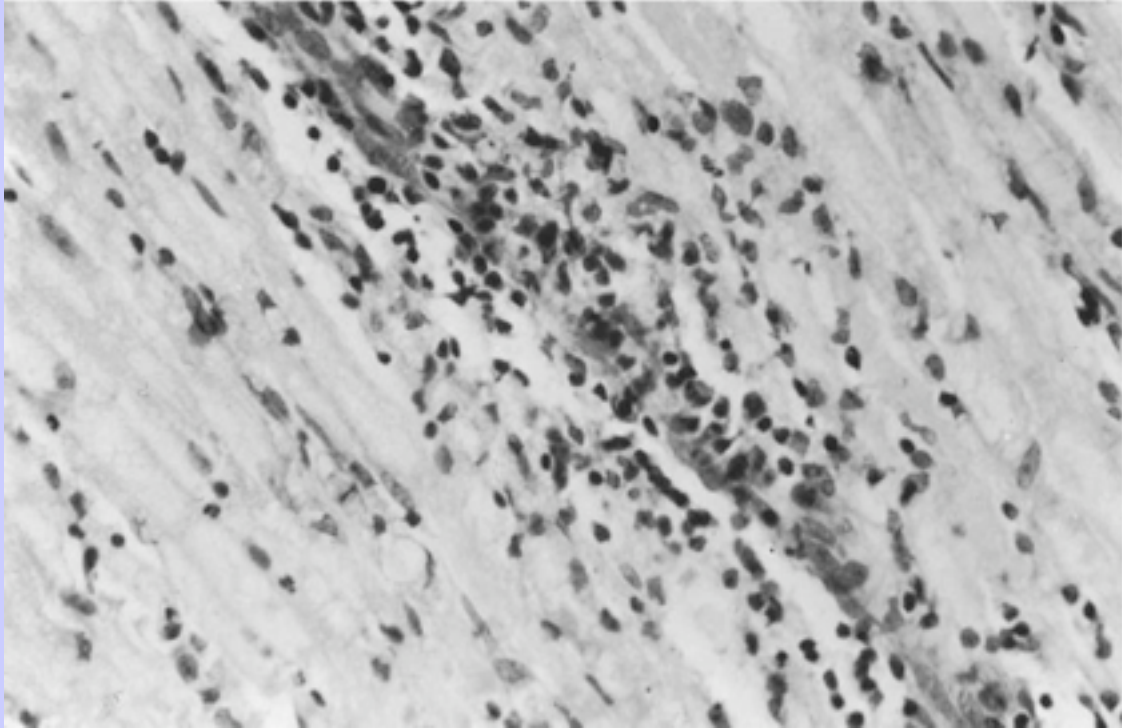
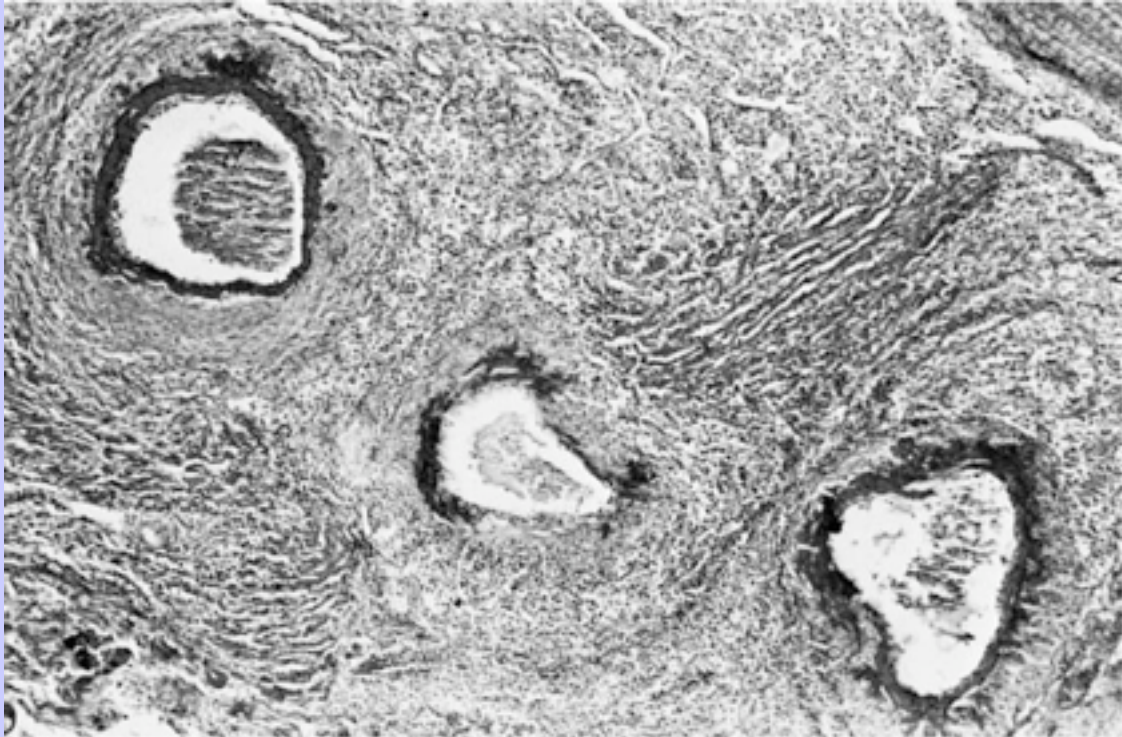


FIGURE 32-4 Meningeal arteries from a dog with meningeal arteritis. Note the periarteritis with fibrinoid necrosis. Original magnification $\times 125$. (From Harcourt RA: Vet Rec 102:519-522, 1978.)



severe cases the dog may develop flaccid quadriplegia and lose the ability to swallow, bark, or breathe. The disease is, however, self-limiting, and if respiration is not impaired the prognosis is good. Dogs usually recover completely. Affected nerves show demyelination and axonal degeneration with macrophage infiltration. An acute polyneuritis similar to coonhound paralysis has also been described following vaccination of dogs with rabies or other vaccines.

Coonhound paralysis and postvaccinal polyneuritis both closely resemble Guillain-Barré syndrome in humans. This syndrome may follow upper respiratory tract infection, gastrointestinal disease, or even vaccination. It is mediated by autoantibodies against peripheral nerve glycolipids. Management of Guillain-Barré syndrome is by plasmapheresis and intravenous immunoglobulins. Steroids are ineffective. Veterinarians treating canine polyneuritis have traditionally administered corticosteroids, but their effectiveness is unclear.

If sciatic nerve tissue is used to immunize experimental dogs, it provokes experimental allergic neuritis. After a latent period of 6 to 14 days, the animals develop an ascending polyneuritis and gradual paralysis ([Figure 32-3](#)). The disease is due to peripheral nerve demyelination resulting from autoimmune attack.

32.3.3 Steroid-Responsive Meningitis-Arteritis

Corticosteroid-responsive meningitis is characterized by sterile inflammation of the meningeal arteries and meningitis. Affected dogs show anorexia, fever, lameness, and listlessness followed by progressive spinal

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rigidity; hyperesthesia; generalized, cervical, or spinal pain; ataxia; seizures; and behavioral changes. These dogs may have concurrent immune-mediated poly-arthritis. Administration of prednisolone leads to rapid clinical improvement. Once the disease is in remission, the dose should be gradually reduced to the minimum necessary to prevent relapses. It may not be possible to discontinue treatment completely. Large dogs, such as Boxers, German Shepherds, or Bernese Mountain Dogs, are commonly affected, although the disease has also been reported in beagles.

The cerebrospinal fluid of these animals contains high immunoglobulin A (IgA) and CXCL8 levels and mature neutrophils. Serum IgA is also elevated. About 30% of these dogs have a positive LE cell test but no detectable antinuclear antibody activity (see [Chapter 33](#)). On necropsy, the spinal meningeal arteries show fibrinoid degeneration, intimal or medial necrosis, and hyalinization, and are infiltrated with lymphocytes, plasma cells, macrophages, and a few neutrophils ([Figure 32-4](#)). Complete obliteration of the blood vessel lumina may occur, while rupture and thrombosis of inflamed vessels may lead to hemorrhage, compression, and infarction.

Immune-mediated vasculitis is usually associated with immune complex deposition and neutrophil infiltration in blood vessel walls. However, in the meningitis described previously, the cellular infiltration may not contain neutrophils. In the beagle cases, no immunoglobulin deposits were detected in the lesions although numerous IgG-containing plasma cells were present in the leptomeninges and in the walls of affected vessels. This may be an autoimmune disease involving local production of IgA.

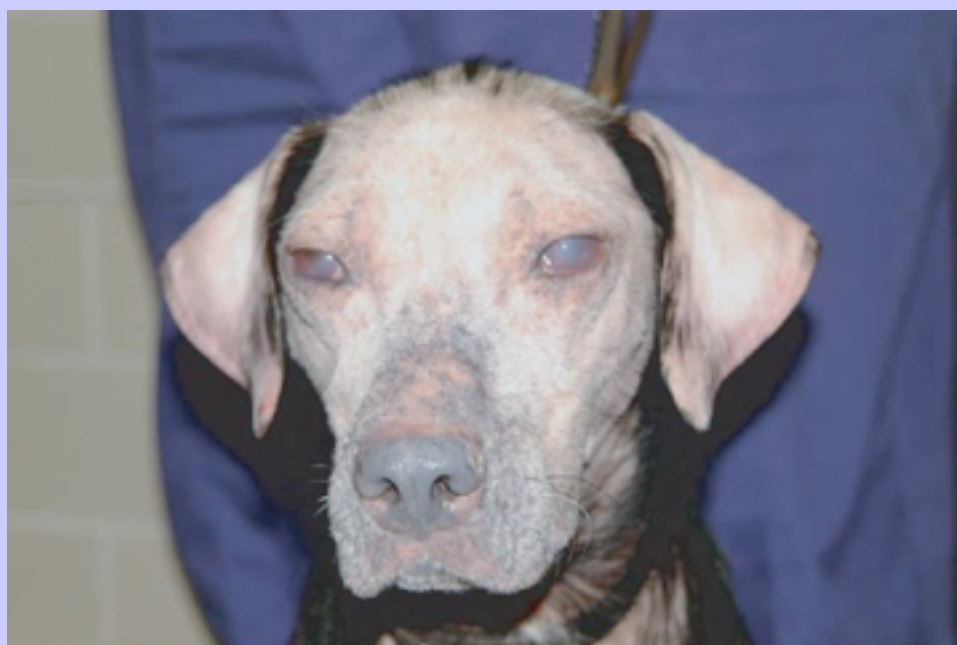
32.3.4 Degenerative Myelopathy

Dogs with degenerative myelopathy show progressive ataxia affecting the hindlimbs until they can no longer

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FIGURE 32-5 A case of uveodermatological syndrome. Note ocular clouding, alopecia, and depigmentation of the nasal planum. (Courtesy Drs. Robert Kennis, Joan Dziezc, and Larry Wadsworth.)



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walk. Forelimb problems eventually develop, and dogs die 6 to 12 months after disease onset. Affected dogs experience widespread demyelination and loss of axons in the thoracolumbar region. The cause of the disease is unknown, but some investigators believe it to be immune mediated. Affected dogs have circulating immune complexes, depressed lymphocyte mitogenic responses, and deposits of IgG and C3 in the lesions and nearby normal tissues. Boxer and Newfoundland dogs affected with inflammatory myopathies have circulating autoantibodies to sarcolemmal autoantigens. It is not clear whether these autoantibodies are a cause or effect of the myopathy. However, detection of these antibodies may be a useful diagnostic test.

32.3.5 Cerebellar Degeneration

Cerebellar degeneration has been observed in Coton de Tulear puppies. It is associated with a depleted granular cell layer and microglial cell activation caused by T cell destruction of granular cells.

32.4 AUTOIMMUNE EYE DISEASE

32.4.1 Equine Recurrent Uveitis

The most common cause of blindness in horses is equine recurrent uveitis (or periodic ophthalmia). Horses have recurrent attacks of uveitis, retinitis, and vasculitis. In acute cases they have blepharospasm, lacrimation, and photophobia. Each attack gets progressively more severe and gradually spreads to involve other eye tissues until complete blindness results. The eye lesions are infiltrated with Th1 cells and neutrophils with extensive fibrin and C3 deposition. The major autoantigen implicated is the interphotoreceptor retinoid-binding protein with subsequent epitope spreading to the S protein. Affected horses also have circulating antibodies to *Leptospira interrogans*. The titer of these antibodies tends to rise during a flare-up of the lesion and drop while in remission. If horses are immunized with either equine cornea or certain serovars of killed *L. interrogans*, they develop corneal opacity 10 days later, when antibodies appear in the bloodstream. Partial antigenic identity exists between equine corneas and these *L. interrogans* serovars. Thus some cases may be due to an autoimmune attack resulting from molecular mimicry with *L. interrogans*. Other cases may be due to infection with *Borrelia burgdorferi* or with the nematode *Onchocerca cervicalis*. Systemic and topical corticosteroid therapy is required to bring the inflammation under control, although the disease usually recurs.

32.4.2 Uveodermatological Syndrome

Uveodermatological syndrome is a sporadic disease of dogs. A similar disease, Vogt-Koyanagi-Harada syndrome, occurs in humans. Affected dogs develop uveitis and skin depigmentation with whitening of the hair (poliosis) and skin (vitiligo). The eye lesions develop first. Thus most animals present with sudden blindness or chronic uveitis. The early lesions vary from a severe panuveitis to a bilateral anterior uveitis. Some dogs may have retinal detachment, and there may be progressive depigmentation of the retina and iris. Depigmentation of the hair and skin gradually follows the onset of the eye lesions. It may be generalized, involving the eyelids, nasal planum, lips, scrotum, and foot pads ([Figure 32-5](#)). These depigmented areas may become ulcerated and crusted.

Histological examination shows a diffuse infiltration of the uveal tract with lymphocytes, plasma cells, and macrophages. Many of the macrophages contain ingested melanin. The skin lesions consist of a mononuclear (macrophages, giant cells, lymphocytes, plasma cells) infiltration of the dermal-epidermal junction ([Figure 32-6](#)). The amount of melanin in the epidermis and hair follicles is greatly reduced.

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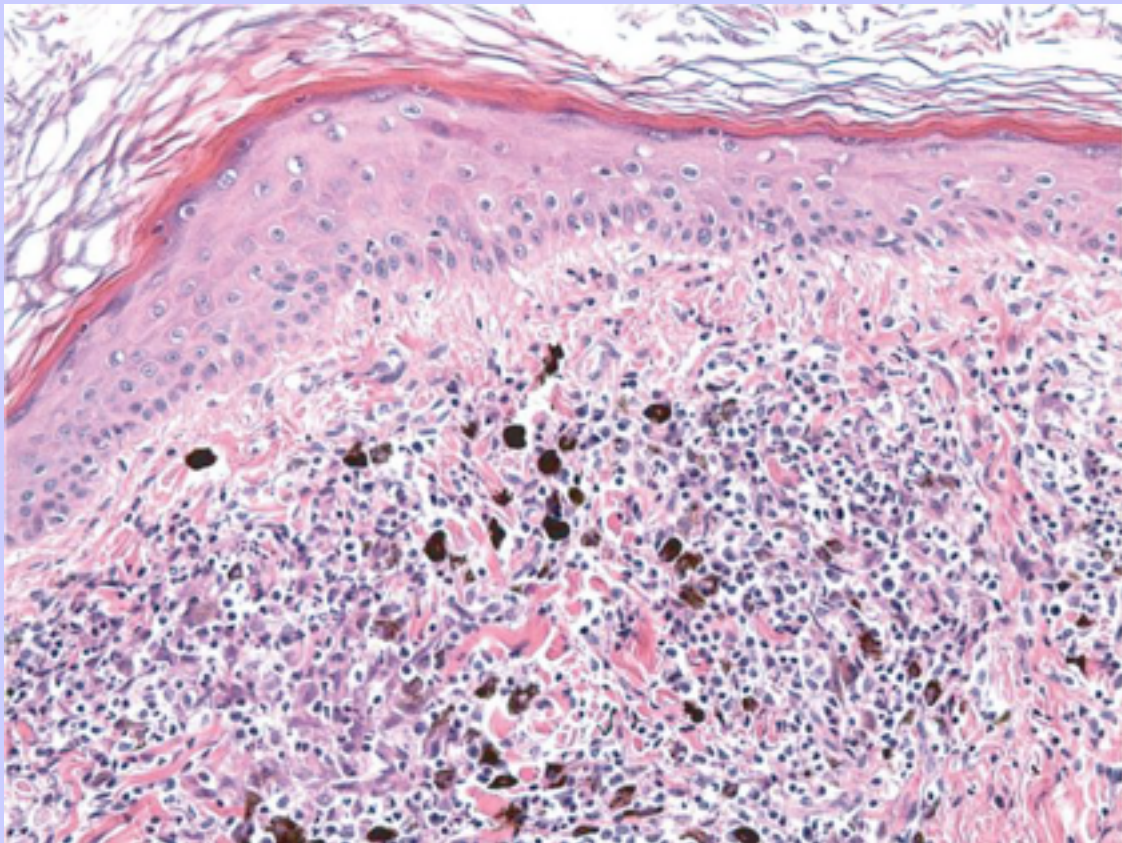
In humans, Vogt-Koyanagi-Harada syndrome is believed to be a result of an autoimmune response against melanocytes. In dogs no consistent immunological abnormalities have been observed.

Management of the eye lesions with ocular glucocorticoids and of the skin lesions with systemic corticosteroids has been beneficial, although the disease may recur when therapy is terminated. Azathioprine may also be given if glucocorticoids are insufficient to stop disease progression.

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FIGURE 32-6 A histological section of skin from a case of uveodermatological syndrome. Note the major lymphocyte infiltration associated with the skin melanocytes. The destruction of these melanocytes leads to depigmentation. (Courtesy Dr. Joanne Mansell.)



32.5 AUTOIMMUNE REPRODUCTIVE DISEASES

If the testes are damaged so that hidden antigens are released, an autoimmune response may exacerbate the orchitis. Experimentally, autoimmune orchitis may be produced in male animals by injection of testicular extracts emulsified in Freund's complete adjuvant. Autoantibodies to sperm may also be detected in the serum of some animals following injury to the testes or long-standing obstruction of the seminiferous ducts. For example, dogs infected with *Brucella canis* have chronic epididymitis and become sensitized by sperm antigens carried to the

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circulation after phagocytosis by macrophages. These sperm antigens stimulate the production of IgG or IgA autoantibodies. The auto-antibodies can agglutinate and immobilize sperm, causing infertility.

In stallions and cows, antisperm autoantibodies may be associated with reduced fertility or infertility. In certain lines of black mink, 20% to 30% of the older males are infertile as a result of high levels of antisperm antibodies. The animals have a monocytic orchitis, and immune complexes are deposited along the basal lamina of the seminiferous tubules.

Dermatologists recognize an autoimmune dermatitis in which intact female dogs develop a hypersensitivity to endogenous progesterone or estrogen. The disease presents as a bilaterally symmetrical, intense pruritus, erythema, and papular eruption. Its development usually coincides with estrus or pseudopregnancy. Corticosteroid treatment may have little effect, but testosterone may help.

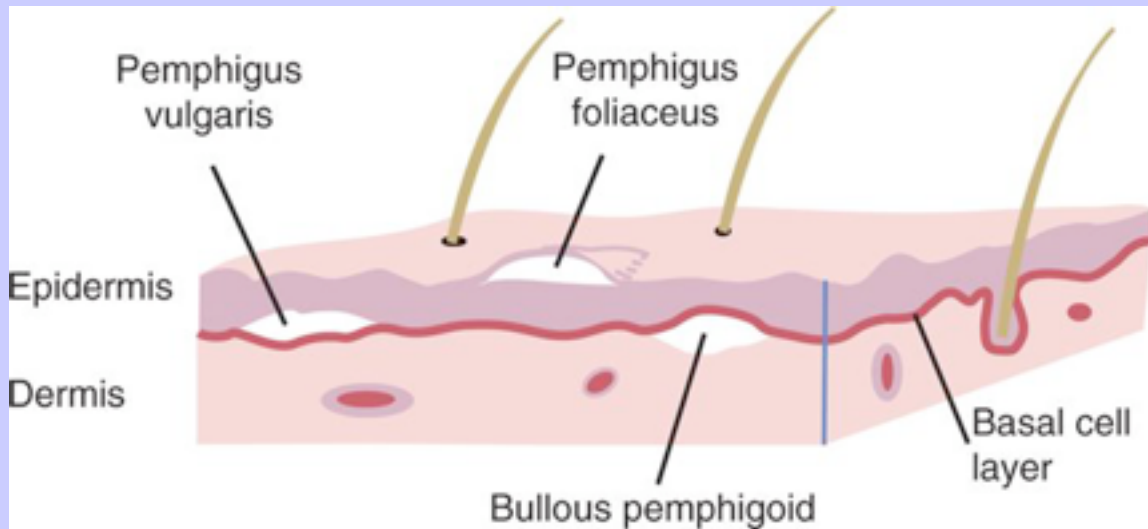
Recently there has been an increased interest in production-enhancing vaccines. These vaccines commonly interfere with normal hormone production or reproductive behavior by inducing an autoimmune response. Thus a vaccine designed to neutralize production of gonadotropin-releasing hormone effectively castrates male animals. Its use results in improved meat quality, faster growth, and reduced aggressive behavior. This vaccine is also used to castrate male pigs and block the production of steroids associated with boar taint, the offensive odor associated with boar meat. Similar vaccines may be used as contraceptives. Thus if dogs are immunized with bovine or ovine luteinizing hormone (LH), the autoantibodies produced may neutralize their own LH. Similarly, it is possible to produce autoantibodies that neutralize LH-releasing hormone. As a result, the reproductive cycle is abolished in females and testicular, epididymal, and prostatic atrophy occurs in male dogs, leading to sterility. Other experimental immunocontraceptive vaccines have been directed against prostaglandin F_{2a} , reproductive steroids, the LH receptor, and zona pellucida protein.

Sheep immunized with polyandroalbumin (androstenedione-7-carboxyethyl thioester linked to human serum albumin) have about 23% more lambs than untreated sheep. The ewes are given two doses of this vaccine before lambing. It is believed that the vaccine induces autoantibodies that reduce serum androstenedione levels.

32.6 AUTOIMMUNE SKIN DISEASES

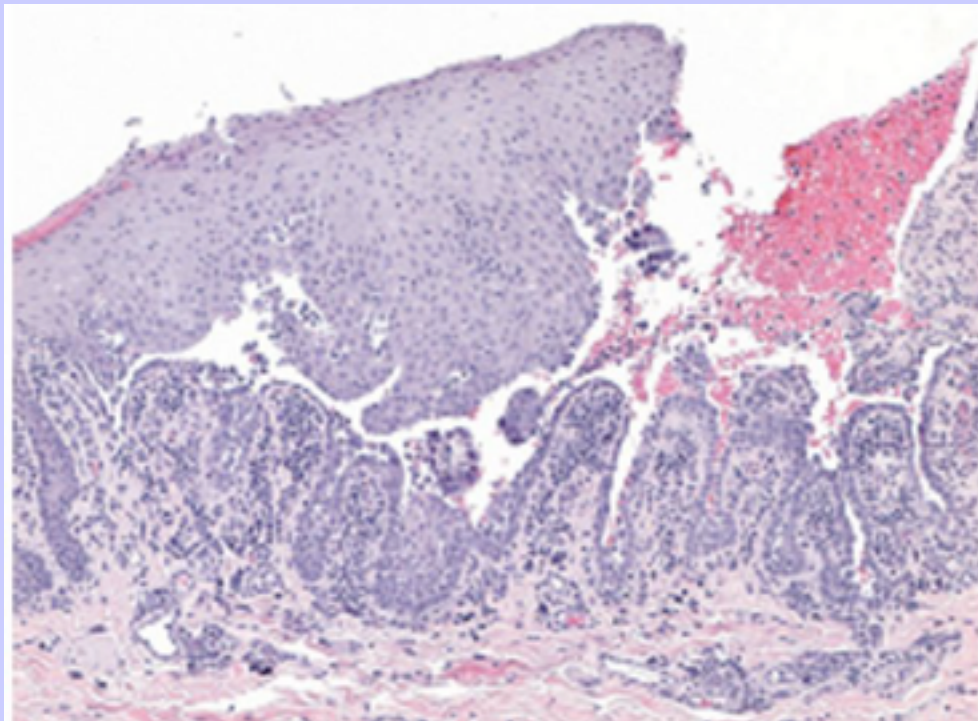
Many different autoimmune skin diseases are recognized. These diseases may affect hair follicles, basal keratinocytes, or the skin basement membrane. While hair follicle disease can lead to alopecia, diseases involving basal keratinocytes or basement membranes are often characterized by cell separation within the skin and the consequent development of bullae (blisters or vesicles) ([Figure 32-7](#)). As a result,

FIGURE 32-7 The differential histology of the autoimmune skin diseases. Note the location of the vesicle in relation to the epidermis.



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FIGURE 32-8 A section of an oral lesion of pemphigus vulgaris in a dog. Note the cleft formation at the base of the epidermis accompanied by extensive cellular infiltration. (Courtesy Dr. Joanne Mansell.)



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dermatologists use the terms *pemphigus* or *pemphigoid* to describe them, after the Greek word *pemphix* meaning “blister.”

32.6.1 Hair Follicle Diseases

32.6.1.1 Alopecia Areata

Alopecia areata is an autoimmune disease characterized by inflammatory hair loss. It has been reported in humans, other primates, dogs, cats, horses, and cattle. In dogs it is a rare disease. The alopecia starts locally, often on the head, but may spread to involve the entire body. It is often symmetrical. The hair follicles are infiltrated with CD4⁺ and CD8⁺ T cells and Langerhans cells. IgG antibodies directed against the lower hair follicles can also be detected. C3 or IgM may also be present. The targets of this immunological attack are unclear but may be directed against a protein called trichohyalin located in the inner root sheath of hair follicles. Alopecia areata responds to corticosteroid treatment, but spontaneous hair regrowth also occurs. Other autoimmune diseases that lead to hair loss include pseudopelade. It differs from alopecia areata in the precise location of the inflammatory infiltrate within the hair follicles. Likewise some cases of pemphigus vulgaris (see later) may also be restricted to hair follicles.

32.6.2 Blistering Diseases

Blistering diseases are a group of related skin diseases that have been described in humans, dogs, horses, and cats. They are also known as the pemphigus complex. They are classified according to the location of the lesions within the epidermis. Some lesions develop deep within the epidermis. For example, the most severe form (although very rare) is called pemphigus vulgaris. In this disease, bullae develop in the skin around the mucocutaneous junctions, especially the nose, lips, eyes, prepuce, and anus, and on the tongue and the inner surface of the ear. These bullae rupture readily, leaving weeping, denuded areas that may become secondarily infected. Histological examination of intact bullae shows a separation of the skin cells (acantholysis) in the suprabasal region of the lower epidermis ([Figure 32-8](#)). The acantholysis results from an autoantibody attack on a cell adhesion protein called desmoglein-3. The combination of antibodies with desmoglein-3 activates the proto-oncogene c-myc and leads to keratinocyte hyperproliferation. As a result, the cells above the lesion proliferate and fail to express adhesion proteins, thus permitting the keratinocytes to separate from each other. Eventually this leads to acantholysis and bulla formation.

Pemphigus foliaceus is a vesicular disease where the lesions develop superficially in the epidermis. As a result it is a milder and much more common disease than pemphigus vulgaris. It has been described in humans, dogs, cats, goats, and horses. The bullae are not confined to mucocutaneous junctions or the muzzle. Histology reveals that the bullae formation occurs superficially in the subcorneal region. These bullae are very fragile, rupture easily, and therefore rarely persist. The autoantigen in humans and some, but not all, dogs has been identified as desmoglein-1, a cell adhesion protein found in squamous cell desmosomes. Some cases of canine pemphigus foliaceus develop following the use of antibiotics such as trimethoprim-sulfadiazine, oxacillin, cephalixin, and ampicillin. They appear to result from the binding of drug thiol groups to cell membranes.

A mild variant of pemphigus foliaceus is pemphigus erythematosus. The lesions in pemphigus erythematosus tend to be confined to the face and ears and are very similar to those of systemic lupus erythematosus. Indeed, some dogs with pemphigus erythematosus may have antinuclear antibodies in their serum. Panepidermal pustular pemphigus (pemphigus vegetans) is another rare and mild variant of pemphigus foliaceus in which papillomatous proliferation of the base of the bullae occurs on healing.

A fifth form of pemphigus, called paraneoplastic pemphigus, is seen in humans and has been recorded in a dog. It develops in association with lymphoid or solid tumors. It resembles pemphigus vulgaris, but multiple autoantibodies against skin antigens are present.

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Direct immunofluorescent examination of pemphigus lesions reveals immunoglobulins deposited on the intercellular cement in a typical “chicken-wire” pattern ([Figure 32-9](#)).

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It is important to differentiate between the forms of pemphigus for prognostic reasons. Pemphigus vulgaris has a poor prognosis: treatment tends to be unsatisfactory and the lesions are persistent. In contrast, pemphigus foliaceus is milder and the results of treatment may be more satisfactory. Treatment of pemphigus primarily involves the use of glucocorticosteroids. In refractory cases, azathioprine, cyclophosphamide, chlorambucil, cyclosporine, or gold salts such as aurothioglucose may be of assistance. As with other autoimmune diseases, the disease often recurs when treatment is stopped.

32.6.3 Skin Basement Membrane Diseases

A second set of blistering diseases is associated with the development of autoantibodies against components of the skin basement membrane. Several of these have been identified in dogs and other domestic animals. They include bullous pemphigoid, linear IgA dermatosis, and epidermolysis bullosa acquisita.

32.6.3.1 Bullous Pemphigoid

Bullous pemphigoid is a rare skin disease that resembles pemphigus vulgaris. Collies, Shetland Sheepdogs, and Dobermans appear to be predisposed to this disease. It has also been described in humans, pigs, horses, and cats. Multiple bullae develop around mucocutaneous junctions and in the groin and axillae. However, the disease differs from pemphigus vulgaris in that the bullae develop in the subepidermis (and are therefore less likely to rupture). They tend to be filled with fibrin as well as mononuclear cells or eosinophils, and they heal spontaneously ([Figure 32-10](#)). Bullous pemphigoid results from the development of autoantibodies against type XVII collagen. This molecule is located in the hemidesmosomes, the structures that attach basal keratinocytes to the basement membrane ([Figure 32-11](#)). The deposition of IgG on the basement membrane may be demonstrated by immunofluorescence that reveals intense linear staining. The prognosis of bullous pemphigoid is usually poor, but mild cases may recover after treatment with corticosteroids. More commonly, aggressive treatment, such as high doses of prednisolone supplemented if necessary with cyclophosphamide, azathioprine, and chlorambucil, may be required. Some dogs may develop a bullous pemphigoid-like disease in response to autoantibodies against the basement membrane protein laminin-5.

32.6.3.2 Linear IgA Dermatitis

Another group of skin diseases is characterized by the deposition of IgA in the lamina lucida of the skin basement membrane. One such disease, called dermatitis herpetiformis, has been recorded in a Beagle, whereas a linear IgA dermatosis has been recorded in Dachshunds. Both diseases present with pruritic pustular and papular lesions, resembling pyoderma, with

FIGURE 32-9 Direct immunofluorescence of a section of normal dog skin that has been incubated in serum from a dog with pemphigus vulgaris. The intercellular cement is stained. (Courtesy Dr. K. Credille.)

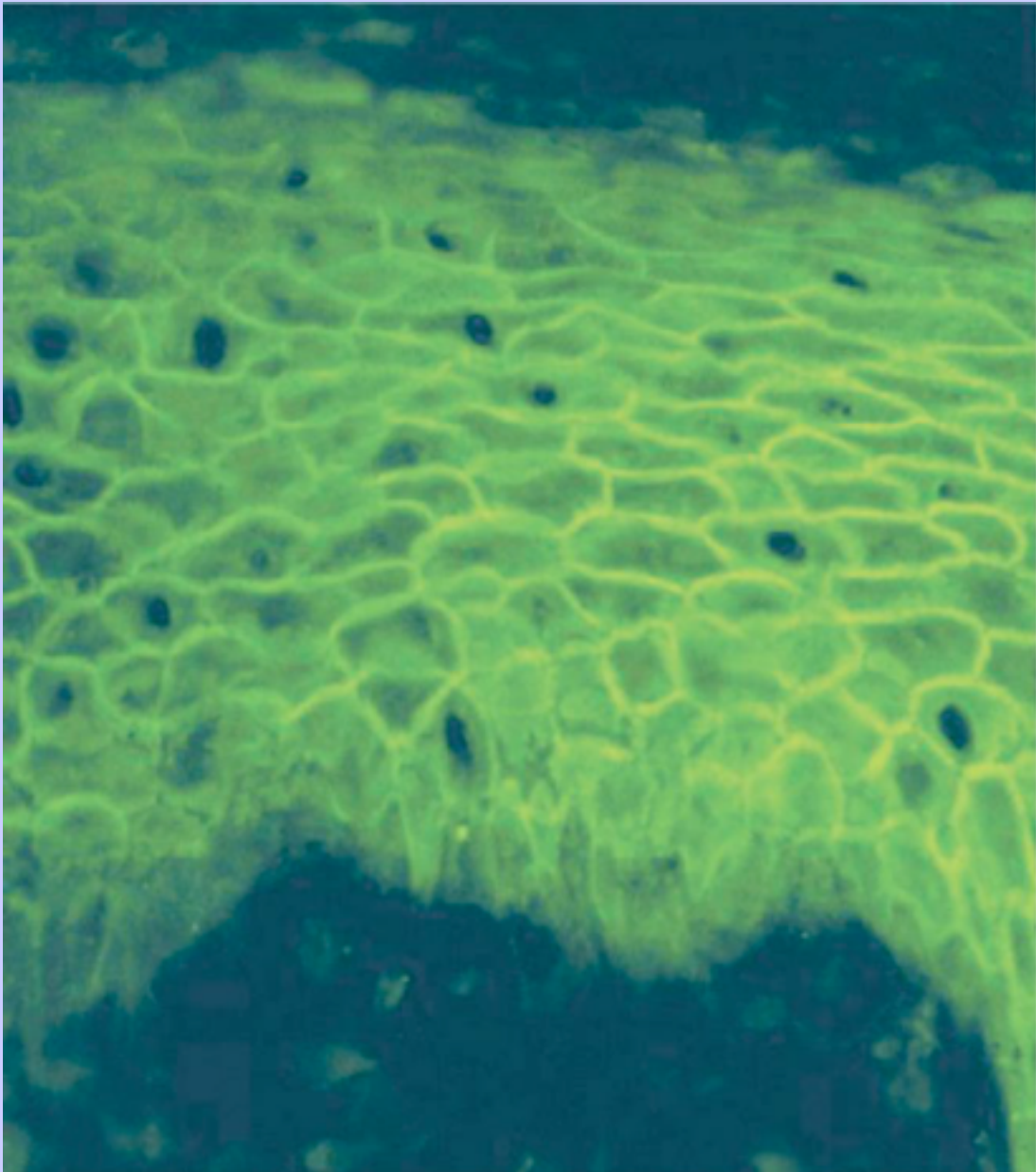
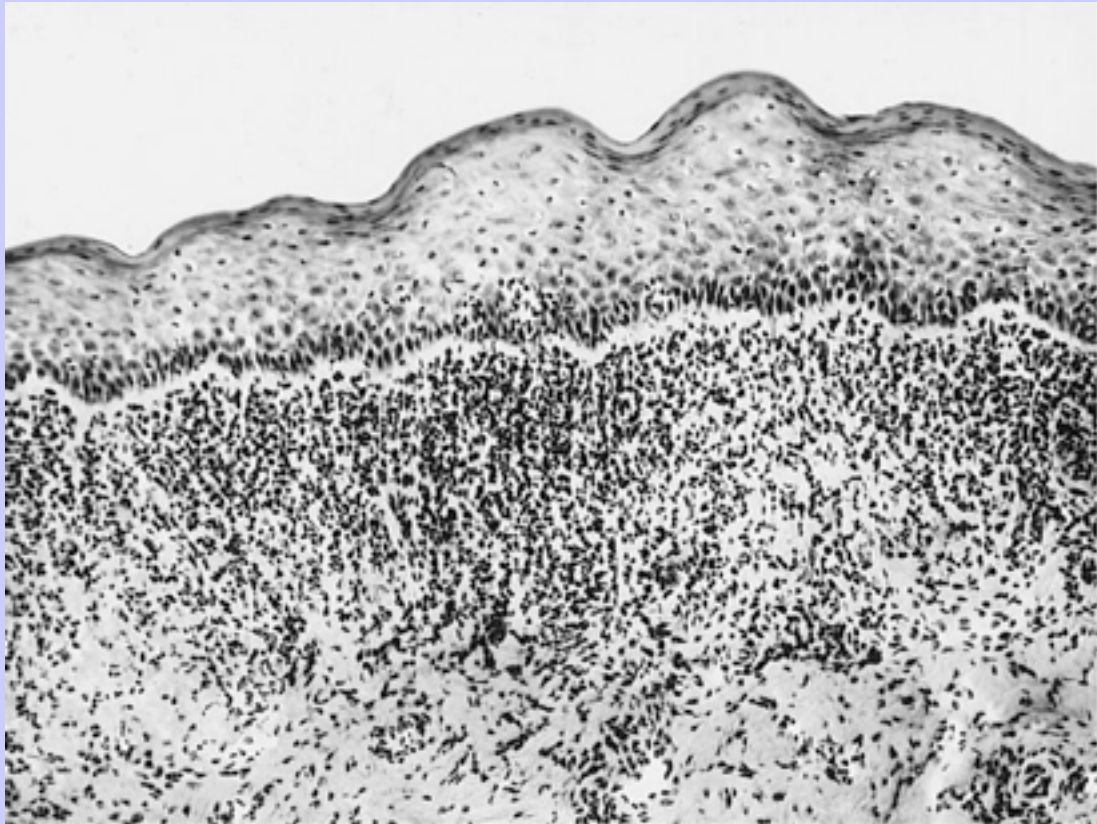
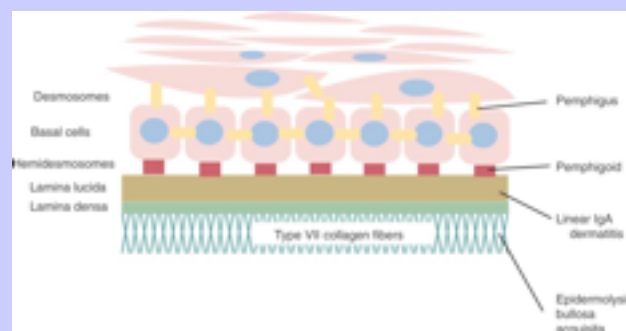


FIGURE 32-10 A section from an oral lesion of bullous pemphigoid in a dog. Note that the cleft is formed below the epidermis. Inflammatory cells are present in the superficial dermis and, to a lesser extent, in the epithelium. (From Bennett D, Lauder IM, Kirkham D, McQueen A: *Vet Rec* 106:497, 1980.)



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FIGURE 32-11 The structures of the skin showing the major structural features that can act as autoantigens.



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eosinophil-filled subepidermal vesicles. The autoantigen has been identified as a processed extracellular form of collagen XVII. The drug dapsone has been recommended as the specific treatment for these diseases.

32.6.3.3 Epidermolysis Bullosa Acquisita

A generalized skin disease characterized by severe blistering and ulcerative lesions has been identified in a Great Dane. The vesicles originate from erythematous areas on the skin and rapidly progress to ulcers. There is generalized urticaria, oral ulceration, and eventually cutaneous sloughing. A localized variant of the disease has been observed in German Short-Haired Pointers. The dermis and epidermis separate and neutrophils accumulate within the superficial dermis. The neutrophil infiltration may eventually result in microabscess formation. Secondary changes include ulceration, necrosis, and bacterial infection. Affected animals develop IgA and IgG autoantibodies against the anchoring fibrils of the lower basement membrane (lamina densa). These autoantibodies are specific for type VII collagen and distinctly different from those responsible for bullous pemphigoid. Immunosuppressive therapy may be of benefit, although secondary bacterial infection can cause difficulties.

32.6.4 Relapsing Polychondritis

A disease involving autoimmunity against type II cartilage has been described in humans and in cats. The animals present with bilateral curling of the ears and ocular changes. The cartilage is infiltrated with plasma cells and lymphocytes.

32.7 AUTOIMMUNE NEPHRITIS

Horses may develop autoantibodies to glomerular basement membranes, which may provoke glomerulonephritis and renal failure. Immunofluorescence studies of affected kidneys show that the basement membrane is evenly coated with a smooth, linear deposit of immunoglobulin. The autoantibodies may provoke proliferation of the glomerular epithelial cells and epithelial crescent formation. A necrotizing encephalitis associated with an anti-glomerular basement membrane glomerulonephritis has been observed in a West Highland White Terrier.

32.8 IMMUNE-MEDIATED HEMOLYTIC ANEMIA

Autoantibodies to red blood cell antigens provoke their destruction and cause autoimmune hemolytic anemia (IMHA). These hemolytic anemias are well recognized in humans and dogs and have been recorded in cattle, horses, cats, mice, rabbits, and raccoons.

Affected dogs are anemic. Pallor, weakness, and lethargy are accompanied by fever, icterus, and hepatosplenomegaly. The anemia may be associated with tachycardia, anorexia, vomiting, or diarrhea. Clinical signs are contingent on the speed of development of the disease, its severity, and the mechanism of red cell destruction. This destruction may result from intravascular hemolysis (destruction within the bloodstream) mediated by complement or, much more commonly, by removal of antibody-coated red cells by the macrophages of the spleen and liver (extravascular hemolysis) ([Figure 32-12](#)). In dogs the disease occurs more often in females (2 : 1 ratio) and in spayed dogs. The average age of onset is around 4 to 5 years. There is evidence for a genetic predisposition to IMHA in some animals (see [Table 32-1](#)), with Cocker Spaniels and Miniature Schnauzers being at increased risk. The causes of IMHA are unknown, although some cases may be attributable to alterations in red cell surface antigens induced by drugs or viruses. In dogs the autoantibodies are primarily directed against red cell glycoporphins, the cytoskeletal protein spectrin, and the membrane anion exchange protein CD233 (band 3). About

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one third of cases of IMHA are associated with other immunological abnormalities such as systemic lupus (see [Chapter 33](#)) or autoimmune thrombocytopenia or with the presence of lymphoid and other tumors. Its onset may be associated with obvious stress such as vaccination (see [Chapter 21](#)), anaplasmosis, viral disease, or hormonal imbalances as in pregnancy or pyometra.

IMHAs in dogs are classified according to the antibody class involved, the optimal temperature at which the autoantibodies react, and the nature of the hemolytic process ([Table 32-2](#)).

32.8.1

Class I

This is caused by autoantibodies that agglutinate red cells at body temperature. The agglutination may be seen when a drop of blood is placed on a glass slide. Both IgG and IgM antibodies are involved. Since IgG does not activate complement efficiently, the red cells are mainly destroyed by phagocytosis in the spleen. In very severe cases, a blood smear may show erythrophagocytosis by neutrophils and monocytes.

32.8.2

Class II

IgM antibodies activate complement and destroy red cells by intravascular hemolysis. This results in hemoglobinemia, hemoglobinuria, icterus, and very severe anemia. Affected dogs are anemic, weak, and possibly jaundiced. Hemoglobin may appear in the urine. Kupffer cells in the liver or in lymph nodes preferentially remove red cells with complement on their surface, so these animals develop hepatomegaly and lymphadenopathy.

32.8.3

Class III

Most cases of IMHA in dogs and cats are mediated by IgG1 and IgG4 antibodies, which bind to red cells at 37° C but do not activate complement or agglutinate the red cells. IgG antibodies can form only short bridges (15 to 25 nm) between cells. As a result, they cannot counteract the zeta potential of the red cells and will not cause direct agglutination. (In contrast, IgM antibodies form long bridges [30 to 50 nm] and so can agglutinate cells despite their zeta potential.) Affected red cells are opsonized and removed by splenic macrophages. Splenomegaly is a consistent feature of class III disease.

FIGURE 32-12 The basic differences between intravascular and extravascular hemolysis.

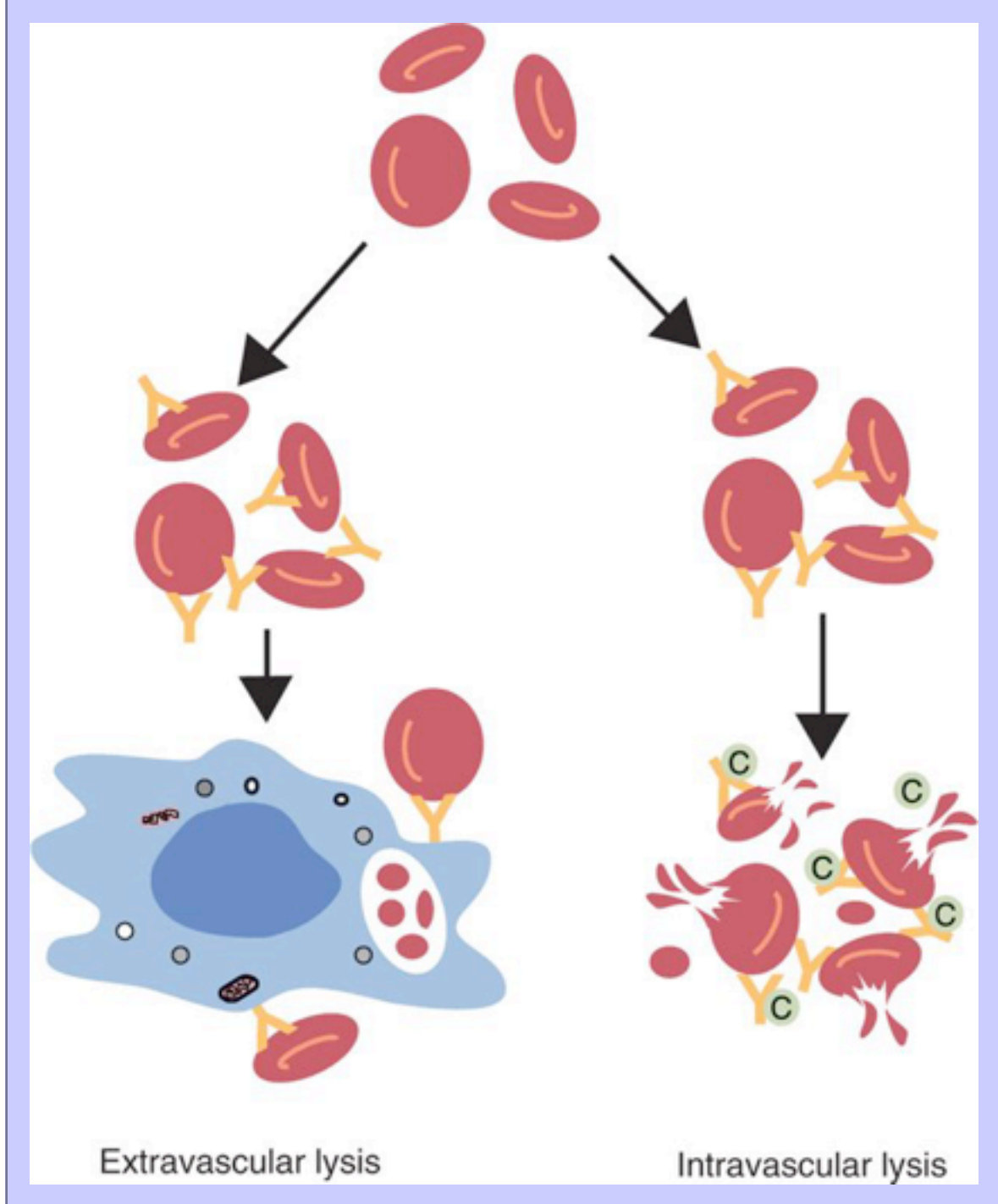


Table 32-2 Classification of Immune-Mediated Hemolytic Anemias

Class	Predominant Antibody	Activity	Optimal Temp. (°C)	Site of Red Cell Removal	Clinical Effect
I	G >> M	Agglutinin	37	Spleen	Intravascular agglutination
II	M	Hemolysin	37	Liver	Intravascular hemolysis
III	G	Incomplete	37	Spleen	Anemia
IV	M	Agglutinin	4	Liver	Cyanosis of extremities
V	M	Incomplete	4	Liver	Anemia

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32.8.4 Class IV

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Some IgM antibodies cannot agglutinate red cells at body temperature but do so only when the blood is chilled. These antibodies are called “cold agglutinins.” They can be detected by cooling blood to between 10° C and 4° C, at which point clumping occurs. The agglutination is reversed on rewarming. As blood circulates through the extremities (tail, toes, ears, and so forth) of affected animals it may be sufficiently cooled to permit hemagglutination within capillaries. This can lead to vascular stasis, blockage, tissue ischemia, and, eventually, necrosis. Affected animals may therefore present with necrotic lesions at the extremities, and anemia may not be a significant feature. As might be anticipated, this form of IMHA is severest in the winter.

32.8.5 Class V

This is mediated by IgM antibodies that will bind red cells when chilled to 4° C but will not agglutinate them. These antibodies can only be identified by an antiglobulin test conducted in the cold. They do not induce necrosis of extremities but can activate complement leading to intravascular hemolysis.

32.8.6 Diagnosis

The hematology of affected animals reflects the severe anemia and a regenerative response by the bone marrow. Blood smears commonly show spherocytes, which are small round red cells that lack a central pale area. These spherocytes result from the partial phagocytosis of antibody-coated red cells. The number of spherocytes in blood is a measure of the intensity of red cell destruction.

To diagnose IMHA associated with the presence of nonagglutinating or incomplete antibodies (classes II, III, V), it is necessary to use a direct antiglobulin test (see [Chapter 38](#)). The red cells of the affected animal are collected in anticoagulant, washed free of serum, and incubated in an antiglobulin serum. The best antiglobulin for these purposes is one with activity against IgM, IgG, and complement. Red cells coated with autoantibody or complement will be cross-linked and agglutinated by the antiglobulin. Occasionally IgM may have a low affinity for the red cells, so it elutes, leaving only complement on their surface. It is important to emphasize that samples for immunological testing should be collected before immunosuppressive therapy begins. It is also important to note that in the cat, most cases of antiglobulin-positive hemolytic anemia are secondary to feline leukemia virus or *Mycoplasma haemofelis* (*Haemobartonella felis*) infections. The disease has a more favorable prognosis in cats than in dogs.

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Treatment for IMHA involves prevention of further hemolysis, treatment of tissue hypoxia, prevention of thromboembolism, and aggressive supportive care. Administration of high doses of corticosteroids reduces phagocytosis of red cells by mononuclear cells and so is the most effective treatment for IgG-mediated disease. Corticosteroids are of much less benefit in the management of intravascular hemolysis mediated by IgM and complement and do not induce significant immunosuppression in these animals. Treated animals may respond within 24 to 48 hours. Corticosteroid treatment may be supplemented with immunosuppressive agents such as cyclophosphamide and/or azathioprine. Ultra-low-dose aspirin may also assist. Splenectomy should only be considered when more conservative therapy has failed. Although splenectomy may be of assistance in cases of refractory class III disease, no controlled trials have confirmed this.

Acute immunologically mediated anemias occur in horses following infection with *Streptococcus fecalis*, in sheep following leptospirosis, in cats with mycoplasmosis (hemobartonellosis), in dogs with babesiosis, and in pigs with eperythrozoonosis. In these cases IgM cold agglutinins clump red cells from normal animals of the same species when chilled. Antibodies to hemoglobin are found in the serum of cattle severely infected with *Arcanobacterium pyogenes*, perhaps as a result of bacterial hemolysis.

IMHA occurs in horses with lymphosarcomas and melanomas. The animals are depressed and pyrexia, and exhibit splenomegaly, jaundice, and hemoglobinuria. Some show red cell autoagglutination. These horses have IgG on their red cells. Dexamethasone treatment can induce remission.

32.8.7

Immune Suppression of Hematopoiesis

In humans, dogs, and cats, autoimmune responses may be directed against hematopoietic stem cells. Thus autoantibodies to erythroid stem cells may cause red cell aplasia, and autoantibodies to myeloid stem cells may provoke an immune neutropenia. In dogs, red cell aplasia has been associated with the presence of IgG, which inhibits erythroid stem cell differentiation. Severe, persistent, immune-mediated neutropenia does occur in dogs. Diagnosis is based largely on excluding other causes of the neutropenia together with a favorable response to steroid and immuno-suppressive therapy. These diseases can only be diagnosed by careful hematological analysis and by demonstration of autoantibodies by immunofluorescence on bone marrow smears. These tests are not easy and have not been validated in domestic species. Affected animals may benefit from high doses of corticosteroids or immunosuppressive therapy.

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32.9

AUTOIMMUNE THROMBOCYTOPENIA

Autoimmune thrombocytopenia (AITP) due to auto-antibodies against platelets has been reported in horses, dogs, and, rarely, cats. Affected animals usually present with multiple petechiae in the skin, gingiva, other mucous membranes, and conjunctiva. Epistaxis, melena, and hematuria may occur. The predominant cause of death in these dogs is severe gastrointestinal hemorrhage. Antibodies against platelet antigens cause extravascular destruction of opsonized platelets in the spleen. As a result, affected animals have unusually low platelet counts and a prolonged bleeding time. The disease is commonly observed in association with IMHA and SLE. The thrombocytopenia seen in animals with multiple myeloma or other lymphoid tumors, in ehrlichiosis, or following certain drug treatments may be due to the nonspecific binding of IgG to platelets. (Drug-induced immune-mediated thrombocytopenia in humans is associated with quinine and vancomycin use.) In dogs, the average age of onset is 6 years. Predisposed breeds include Old English Sheepdogs, Cocker Spaniels, and Poodles. Antibodies to platelets may be measured by direct immunofluorescence on bone marrow aspirates looking for positive staining on megakaryocytes. However, the best test for this purpose is one that measures the release of factor 3 from platelets following exposure to autoantibodies. This may be performed by incubating platelet-rich plasma with a globulin fraction of the serum

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under test and estimating the amount of procoagulant activity released. In about 75% of cases the antibodies are of the IgG class. Most cases of AITP in cats are probably secondary to feline leukemia virus infection.

Corticosteroids are used to treat AITP. Additional immunosuppression with azathioprine or cyclophosphamide may be required for patients who do not respond to corticosteroids. Vincristine may also produce a good clinical response since it binds to platelets and kills macrophages when they phagocytose the antibody-coated platelets. Good results have also been obtained with intravenous human immunoglobulins. Splenectomy may help when other forms of therapy have failed.

32.10 AUTOIMMUNE MUSCLE DISEASE

32.10.1 Myasthenia Gravis

Myasthenia gravis in humans, dogs, and cats is a disease of skeletal muscle characterized by abnormal fatigue and weakness after relatively mild exercise. It results from a failure of transmission of nerve impulses across the motor endplate of striated muscle as a result of a deficiency of acetylcholine receptors. In Jack Russell Terriers, Springer Spaniels, and Fox Terriers, a congenital form of the disease occurs as a result of an inherited deficiency of these receptors. This congenital form is therefore a disease of young dogs.

In adult dogs, however, the acetylcholine receptor deficiency is due to IgG autoantibodies ([Figure 32-13](#)). These antibodies accelerate degradation of the receptors, block the acetylcholine-binding sites, and trigger complement-mediated damage. As a result, the number of available, functional acetylcholine receptors is significantly reduced. Dogs may also make autoantibodies against titin, an intracellular muscle protein and the ryanodine receptor—a Ca^{2+} release channel in striated muscle.

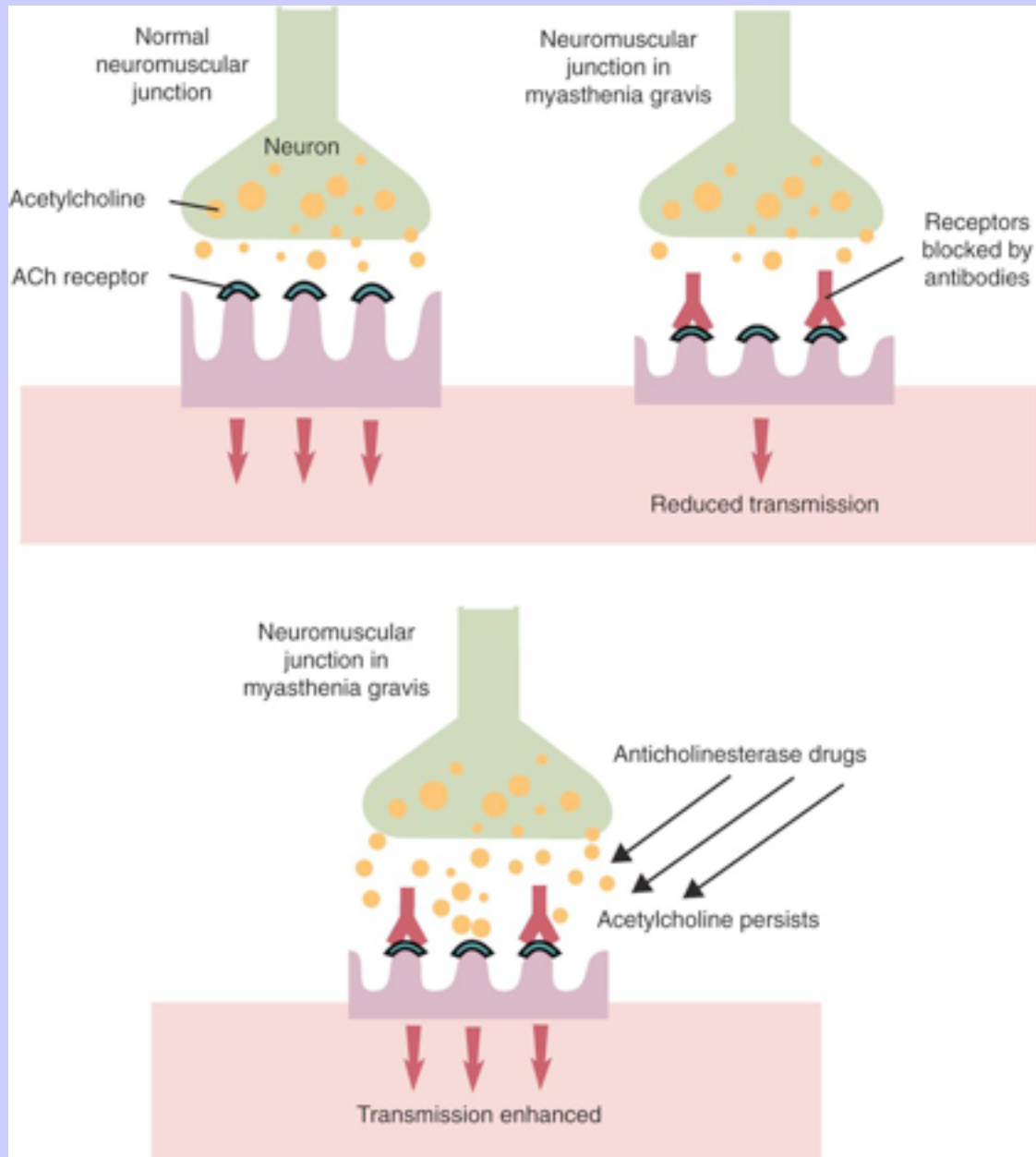
In normal muscles, the binding of acetylcholine to its receptor opens a sodium channel to produce a localized endplate potential. If the amplitude of the endplate potential is sufficient, this will generate an action potential and trigger muscle contraction. The endplate potential from a normal neuromuscular junction is more than sufficient to generate a muscle action potential. In myasthenic junctions, however, the endplate potentials fail to trigger action potentials in many muscle fibers. This is manifested as muscle weakness. Since the amount of acetylcholine released from a nerve terminal usually declines after the first few impulses, repeating the stimulus leads to a progressive increase in weakness as transmission failure occurs at more and more neuromuscular junctions.

The disease may develop in any breed of dog but certain breeds are predisposed to it (see [Table 32-1](#)). Large dogs such as German Shepherds, Golden Retrievers, Labradors, and Dachshunds appear to develop more severe disease. Rottweilers appear to be at low risk. In cats there appears to be a breed predisposition for Abyssinians and related Somalis.

In some animals the thymus may show medullary hyperplasia, germinal center formation, or even a thymic carcinoma, and surgical thymectomy may result in clinical improvement. About 3% of dog cases and 20% of cat cases are associated with the presence of a thymic tumor.

Animals may present with a history of swallowing difficulty, regurgitation, labored breathing, and generalized muscle weakness. Megaesophagus is common. Clinically different disease forms may be recognized. The disease is classified as focal myasthenia gravis when an animal presents with megaesophagus and various degrees of facial paralysis without limb muscle weakness; generalized myasthenia gravis when limb muscle weakness is associated with facial paralysis

FIGURE 32-13 The pathogenesis of myasthenia gravis. Destruction of acetylcholine (ACh) receptors prevents effective neuromuscular transmission. Blockage of cholinesterase activity by anticholinesterase drugs permits acetylcholine to accumulate and so enhances neuromuscular transmission.



and megaesophagus; and acute fulminating myasthenia gravis when the disease rapidly leads to quadriplegia and respiratory difficulty. Almost 60% of cases are generalized or fulminating, while the rest are focal. Without

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treatment about half of affected animals will die whereas the others may show spontaneous remissions. Aspiration pneumonia is the main cause of death in myasthenic dogs.

Administration of a short-acting anticholinesterase drug such as edrophonium chloride (tensilon) leads to a rapid gain in muscle strength. The anticholinesterase, by permitting the acetylcholine to accumulate at the neuromuscular junction, enables the remaining receptors to be stimulated more effectively. Myasthenia gravis is managed with long-acting anticholinesterase drugs such as pyridostigmine bromide or neostigmine methyl sulfate. Dogs may also be immunosuppressed with prednisone or azathioprine or both. However, corticosteroid treatment may result in transient exacerbation of symptoms. Plasmapheresis has been used for short-term therapy to stabilize patients before thymectomy.

32.10.2 Polymyositis

A generalized autoimmune myositis occurs in large dogs such as German shepherds. The disease may be acute or gradual in onset. The animals show progressive muscle weakness not associated with exercise. Changes in laryngeal muscle function lead to a change in the voice. Megaesophagus may lead to dysphagia and, if severe, can result in aspiration pneumonia. Affected animals may develop a shifting lameness. Animals may be febrile and develop leukocytosis and eosinophilia. Biopsies show muscle fiber degeneration, necrosis, and vacuolation, and affected muscles may be infiltrated by lymphocytes and plasma cells. About 50% of affected dogs have antinuclear antibodies or antibodies to sarcolemma or both. Corticosteroids are the treatment of choice. A similar immune-mediated myositis has been recorded in quarter horses. It causes rapid atrophy of the gluteal and epaxial muscles. The affected muscles are infiltrated with macrophages and CD4⁺ lymphocytes with lesser numbers of CD8⁺ cells and B cells. It may be treatable with corticosteroids.

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32.10.3 Autoimmune Masticatory Myositis

Dogs may develop an autoimmune myositis confined to the muscles of mastication. The major antigen recognized is called masticatory myosin binding protein-C. It is found only in masticatory muscle fibers. Affected animals present with pain and atrophy or swelling of the masticatory muscles resulting in difficulty in opening (trismus) or closing the jaw. They may also have eye lesions such as conjunctivitis or exophthalmos. Histology of affected muscles shows inflammatory or degenerative lesions affecting the M2 myofibrils. Myositis with lymphocytes and plasma cells predominates, and some lesions may contain many eosinophils. Myofiber atrophy, perimysial or endomysial fibrosis, and muscle fiber necrosis are consistent features. Immunoglobulins may be detected in biopsies of affected muscles, and circulating antibodies to the M2 myofibrils have been demonstrated by an immunoperoxidase assay. Corticosteroids such as prednisone are used for treatment, but the prognosis is guarded. Cavalier King Charles Spaniels may be especially predisposed to this disease.

32.10.4 Canine Cardiomyopathy

English Cocker Spaniel dogs may develop a cardiomyopathy with antinuclear and antimitochondrial autoantibodies and reduced serum IgA levels. It is associated with a specific C4 allotype (C4 : 4). The autoantigen has not been identified, but in humans some forms of cardiomyopathy are due to autoantibodies directed against the adenine nucleotide translocator of mitochondria.

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32.11 CHRONIC ACTIVE HEPATITIS

Doberman Pinschers may develop an autoimmune hepatitis. The symptoms are typical of liver disease, with anorexia, depression, weight loss, diarrhea, polydipsia, polyuria, icterus, and eventually ascites. The disease commonly presents between 3 and 6 years of age but may have been present subclinically for many years. On histology, the liver shows intense inflammation and scar tissue formation around small hepatic vein branches. The lesions contain lymphocytes, plasma cells, and macrophages. The disease eventually causes progressive fibrosis and destruction of hepatocytes. About half of affected dogs develop antibodies to hepatocyte cell membranes. These antibody-positive dogs have more severe disease than dogs without antibodies. In addition, lymphocytes from about 75% of affected dogs respond to liver membrane proteins in vitro. Hepatocytes from affected dogs, but not from normal Dobermans, express major histocompatibility complex (MHC) class II antigens. This MHC expression correlates with the severity of the disease, while corticosteroid treatment reduces both MHC expression and disease severity. It has been suggested therefore that the disease results from a cell-mediated attack on abnormally expressed MHC molecules or an antigen associated with them.

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³³ CHAPTER 33 The Systemic Immunological Diseases

33.1 KEY POINTS

- Some autoimmune diseases may involve immunological attack on many different organs and tissues at the same time. This probably reflects a major loss of control of the innate and acquired immune systems.
- Systemic lupus erythematosus is a complex autoimmune disease characterized by multiple autoimmune responses together with the presence of autoantibodies to nuclear antigens. Lupus may represent several different disease entities.
- Many different forms of arthritis may also be immunologically mediated. The most significant of these is the erosive joint disease called rheumatoid arthritis. It is associated with the development of autoantibodies to joint components such as collagens and of rheumatoid factor, an autoantibody against immunoglobulin G.
- There is a significant clinical overlap between many of these syndromes making precise diagnosis difficult.

Animals may suffer from complex inflammatory diseases that involve multiple organ systems. In human medicine these have been called “rheumatic” diseases, “connective tissue” diseases, or “collagen” diseases based on outdated views of their pathogenesis. These systemic diseases or syndromes are interrelated and have many overlapping clinical features ([Figure 33-1](#)). One common feature is extensive and uncontrolled inflammatory responses, and it may be useful to consider them to be forms of innate autoimmunity or “autoinflammatory diseases.” Be-cause of their many similarities and overlaps, it is sometimes difficult to come to a definitive diagnosis of the precise syndrome involved.

These innate autoimmune diseases include systemic lupus erythematosus, rheumatoid arthritis, nonerosive arthritides, various vasculitides, dermatomyositis, and Sjögren's syndrome. In humans a large number of inherited inflammatory diseases have been recognized. In many cases these diseases result from inherited defects in the activation of caspases 1 and related molecules. It is predicted that some of these diseases will eventually be recognized in domestic mammal species. Although all these diseases have some form of acquired autoimmune component, they are not simply diseases in which autoantibodies cause tissue destruction. Most are associated with the presence of immune complexes and complement in tissues leading to chronic inflammation. Many appear to result from uncontrolled inflammatory cytokine production or abnormalities in the complement system. Their initiating factors are unknown but may well be infectious

FIGURE 33-1 The interrelationships among the diseases discussed in this chapter. The diagram is somewhat simplified, since polyarthritis may be associated with polymyositis.

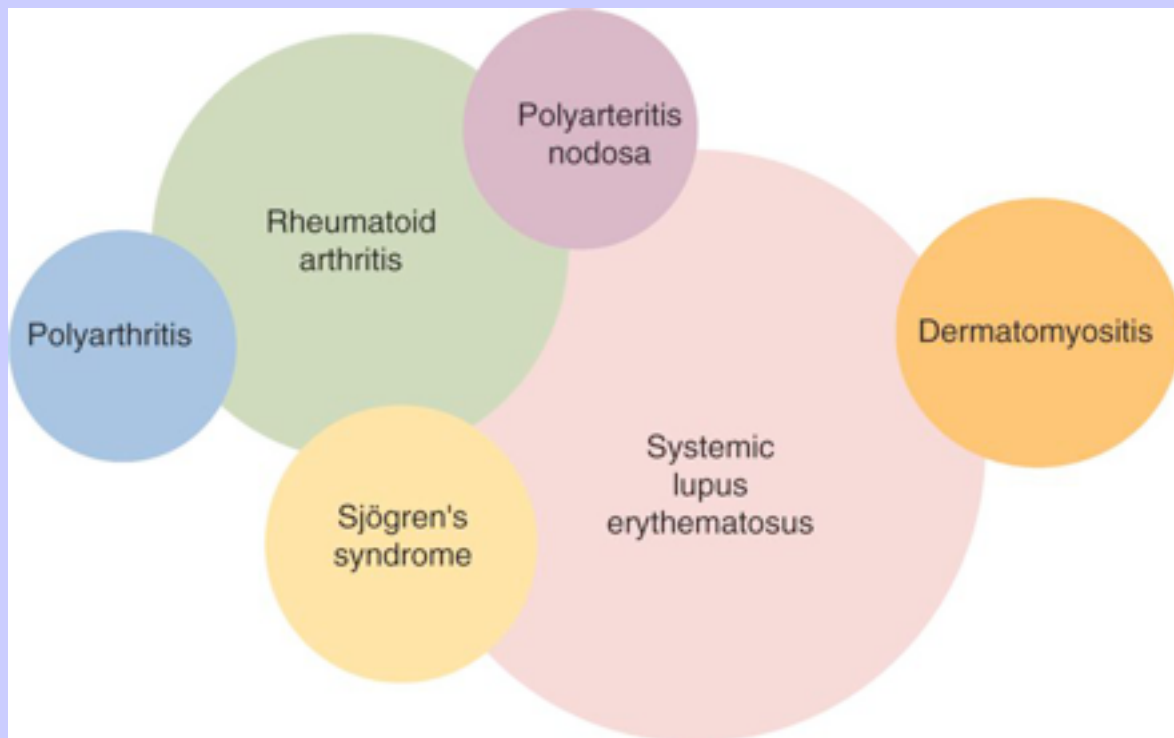
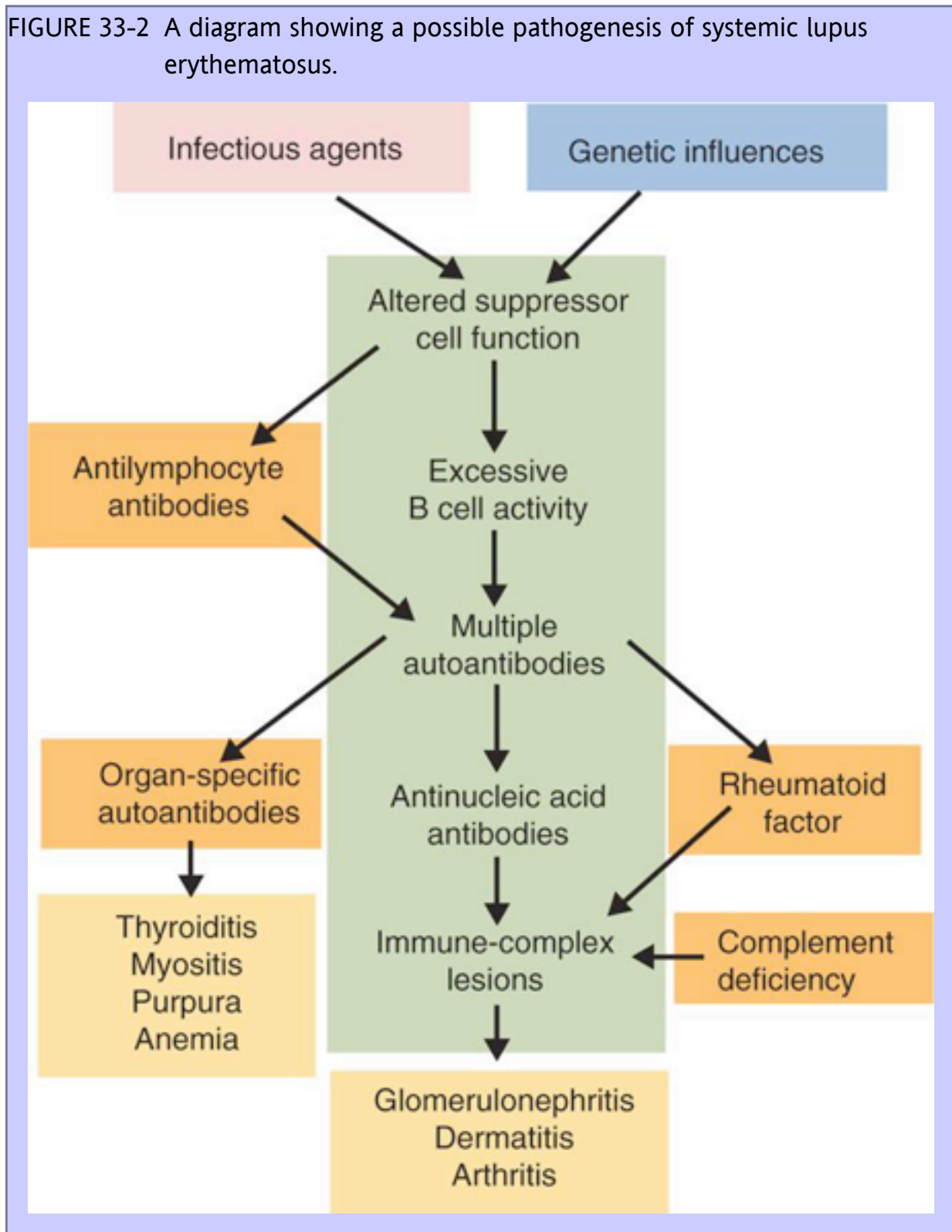


FIGURE 33-2 A diagram showing a possible pathogenesis of systemic lupus erythematosus.



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agents acting through toll-like receptors (TLRs). All exhibit a significant genetic predisposition, commonly with linkage to the major histocompatibility complex (MHC).

33.2 SYSTEMIC LUPUS ERYTHEMATOSUS

Systemic lupus erythematosus (SLE) is a complex dis-ease syndrome (or perhaps even multiple diseases) that has been described in humans, other primates, mice, horses, dogs, and cats. It is characterized by a broad and bewildering diversity of different symptoms and a wide variety of disease courses as symptoms flare and recede over time.

33.2.1 Pathogenesis

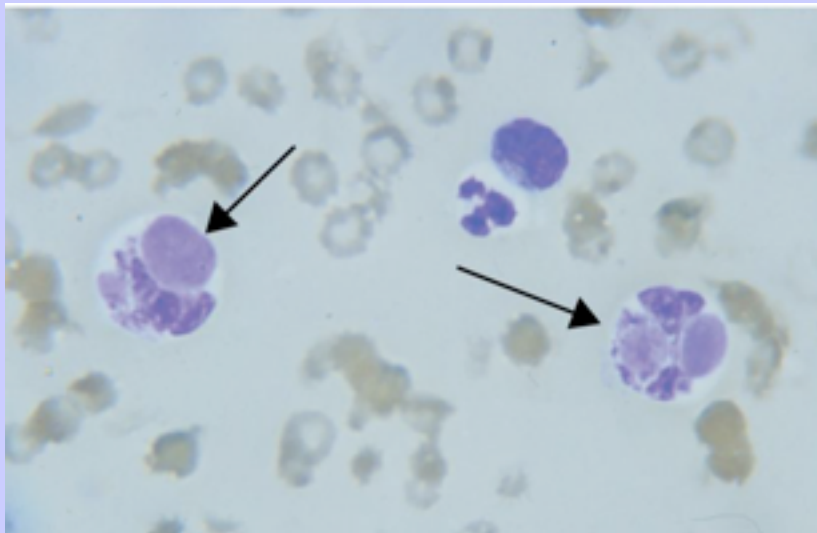
The factors that lead to the development of lupus are complex, multifactorial, and poorly defined. Its development is affected by environmental factors, including infectious agents, drugs, and food, in association with many different genes ([Figure 33-2](#)). Patients develop a variety of autoantibodies, changes in T cell function, defective phagocytosis, and oncogene expression.

One consistent feature of all forms of lupus is the development of autoantibodies against antigens located within the cell nucleus. These antinuclear antibodies (ANAs) are found in 97% to 100% of dogs with lupus as compared with 16% to 20% of normal control animals. About 16 different nuclear antigens have been described in humans. Dogs differ from humans in that they develop autoantibodies not against native, double-stranded DNA, but against nuclear proteins such as histones and ribonucleoproteins. ANAs can cause tissue damage by several mechanisms. They can combine with free antigens to form immune complexes that are deposited in glomeruli causing a membranous glomerulonephritis (see [Chapter 27](#)). They may be deposited in arteriolar walls, where they cause fibrinoid necrosis and fibrosis, or in synovia, where they provoke arthritis. ANAs also bind to the nuclei of degenerating cells to produce round or oval structures called hematxylin bodies in the skin, kidney, lung, lymph nodes, spleen, and heart. Within the bone

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FIGURE 33-3 Two lupus erythematosus cells (*arrows*) from a dog with systemic lupus erythematosus ($\times 1300$).



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marrow, opsonized nuclei may be phagocytosed, giving rise to lupus erythematosus (LE) cells ([Figure 33-3](#)).

In addition to making ANAs, many patients with lupus and related diseases make high levels of auto-antibodies against one or two extracted nuclear antigens. One is called SSA/Ro, a group of at least five intracellular ribonucleoproteins. Antibodies to SSA/Ro are classically found in Sjögren's syndrome. Another major antigen is called SSB/La. This is a nuclear ribonucleoprotein, antibodies against which are found in lupus patients. There appear to be significant and consistent clinical differences between patients producing these two types of autoantibody.

The production of ANAs in lupus may be a result of several possible defects. One possibility is that their TLRs, notably TLR7 and TLR9, lose the ability to discriminate between microbial and self-DNA. If, at the same time, some of their B cells happen to undergo somatic mutation that enables their surface immunoglobulins to bind self-DNA, then all the ingredients necessary for a profound antibody response to mammalian DNA come together.

Bacterial DNA is a potent antigen and immune stimulant. Since mammalian DNA and bacterial DNA have a conserved backbone structure, it is possible that patients with lupus may respond to bacterial infection by producing cross-reactive antibodies that react with mammalian DNA. For example, the New Zealand Black/New Zealand White mouse strain spontaneously develops a lupuslike syndrome when immunized with bacterial DNA by producing cross-reactive antibodies to mammalian dsDNA. These anti-DNA antibodies may form immune complexes and cause arthritis, skin rashes, and vascular disease. Antibody-DNA immune complexes may bind to TLR9 and activate autoreactive B cells by triggering both the TLR and antigen receptors.

A defect in some lupus patients appears to be the impaired clearance of apoptotic cells. Normally, apoptotic cells are removed by phagocytosis by macrophages without causing inflammation. Macrophages from SLE patients, however, show defective phagocytosis of apoptotic cells, which as a result, accumulate in tissues. Nuclear fragments from these cells may be trapped and processed by dendritic cells, thereby triggering autoantibody formation. The defect is most obvious in the skin of affected animals, where UV radiation leads to accumulation of apoptotic cells. Nucleic acids from these cells then act as autoantigens and trigger autoantibody formation. These autoantibodies in turn lead to immune complex deposition and tissue damage.

Although ANAs are characteristic of lupus, many other autoantibodies are produced, suggesting that affected animals may also have abnormal B cells. Affected animals show abnormalities in B cell signal-ing and migration, overexpression of CD154 (CD40L), and enhanced production of interleukin-6 (IL-6) and IL-10. Some experimental mouse models show over-expression of B cell stimulatory molecules by T cells and dendritic cells. It is therefore possible that the production of multiple autoantibodies in lupus is a combined result of defective apoptosis, overstimulation of B cells, and a failure to eliminate self-reactive B cells.

Autoantibodies to red cells induce a hemolytic anemia. Antibodies to platelets induce a thrombocytopenia. Antilymphocyte antibodies may interfere with immune regulation. About 20% of dogs with lupus produce antibodies to immunoglobulin G (IgG) (rheumatoid factors). Antimuscle antibodies may cause myositis, and antimyocardial antibodies may provoke myocarditis or endocarditis. Antibodies to skin basement membrane cause a dermatitis characterized by changes in the thickness of the epidermis, focal mononuclear cell infiltration, collagen degeneration, and immunoglobulin deposits at the dermo-epidermal junction. These deposits form a "lupus band," seen in many other autoimmune skin diseases in addition to lupus ([Figure 33-4](#)). In humans especially, lupus skin lesions are commonly restricted to the bridge of the nose and the area around the eyes since apoptosis is exacerbated by UV radiation in sunlight. The results of this excessive immune reactivity are also reflected in a polyclonal gammopathy, enlargement of lymph nodes and spleen, and thymic enlargement with germinal center formation.

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The great variety of autoantibodies produced in lupus can cause an equally great variety of clinical symptoms. Polyarthrititis, fever, proteinuria, anemia, and skin diseases are the most common abnormalities, but pericarditis, myocarditis, myositis, lymphadenopathy, and pneumonia have also been reported.

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FIGURE 33-4 A lupus band in a section of monkey esophagus. The indirect immunofluorescence assay shows immunoglobulin G deposition on the skin basement membrane. (Courtesy Dr. F. Heck.)

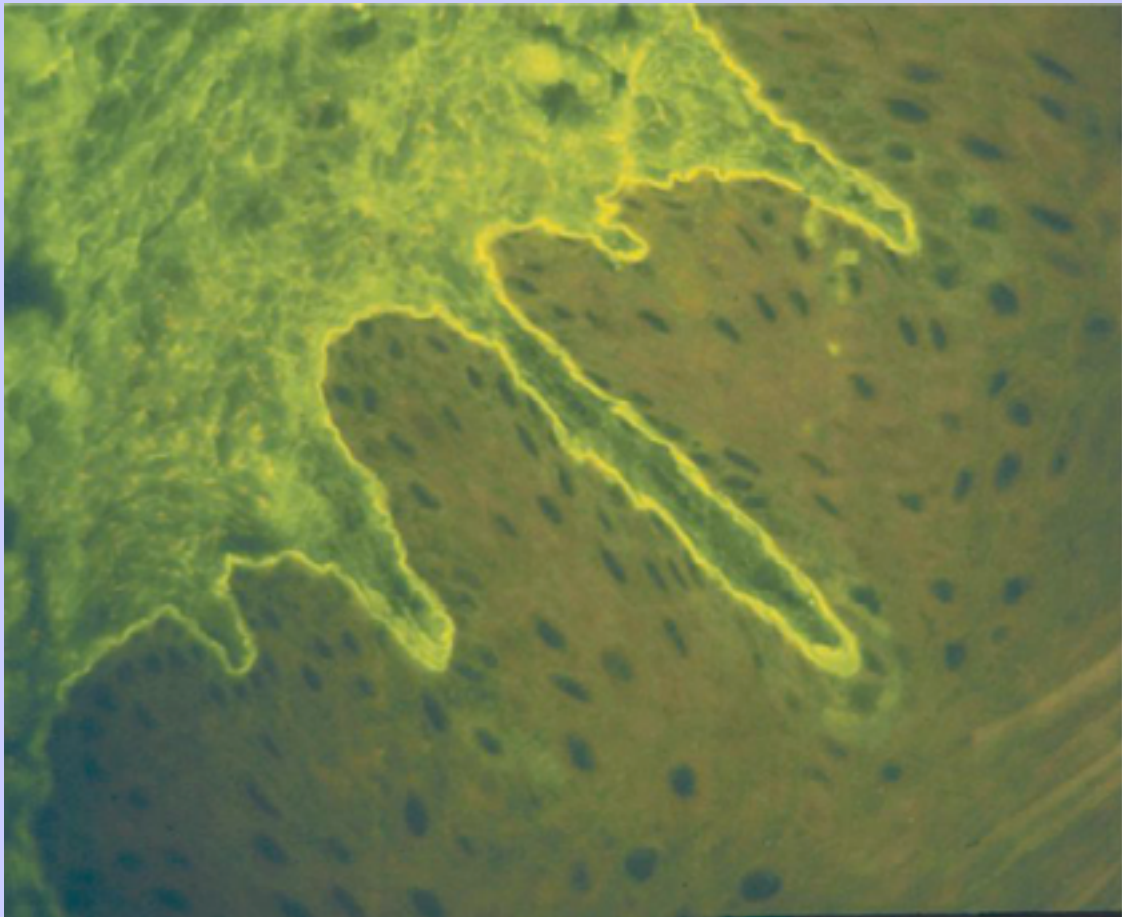


FIGURE 33-5 A filly with systemic lupus erythematosus. Note the generalized alopecia and crusting. (From Geor RJ, Clark EG, Haines DM, Napier PG: *J Am Vet Med Assoc* 197:1489, 1990.)



Although lupus likely involves a failure of apoptosis, leading to activation of autoimmune B cells and multiple autoimmune disorders, its initiating cause remains obscure. There is good evidence for a genetic predisposition in humans, dogs, and mice. It is also associated with an inherited deficiency of certain complement components and certain Fcγ receptors.

Hormones clearly influence the development of the disease, as shown by the fact that women develop lupus about eight times as often as men and because it becomes more severe during pregnancy. Estrogens affect apoptosis through CD95, CD95L, interferon- γ (IFN- γ), and nitric oxide and interfere with B cell tolerance. Virus infections may trigger the syndrome. Thus individuals with lupus commonly have high levels of antibodies to parainfluenza 1 and measles. Myxovirus-like structures have been seen in renal endothelial cells from lupus patients, and type C retroviruses have been isolated from patients and associated with the disease.

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As described in [Chapter 5](#), some lupus patients have a deficiency of the complement receptor CD35. As a result, immune complexes are not bound to red cells or platelets and therefore are not effectively removed from the circulation. These immune complexes may then be deposited in the glomeruli or in joints.

33.2.1.1 Equine Lupus

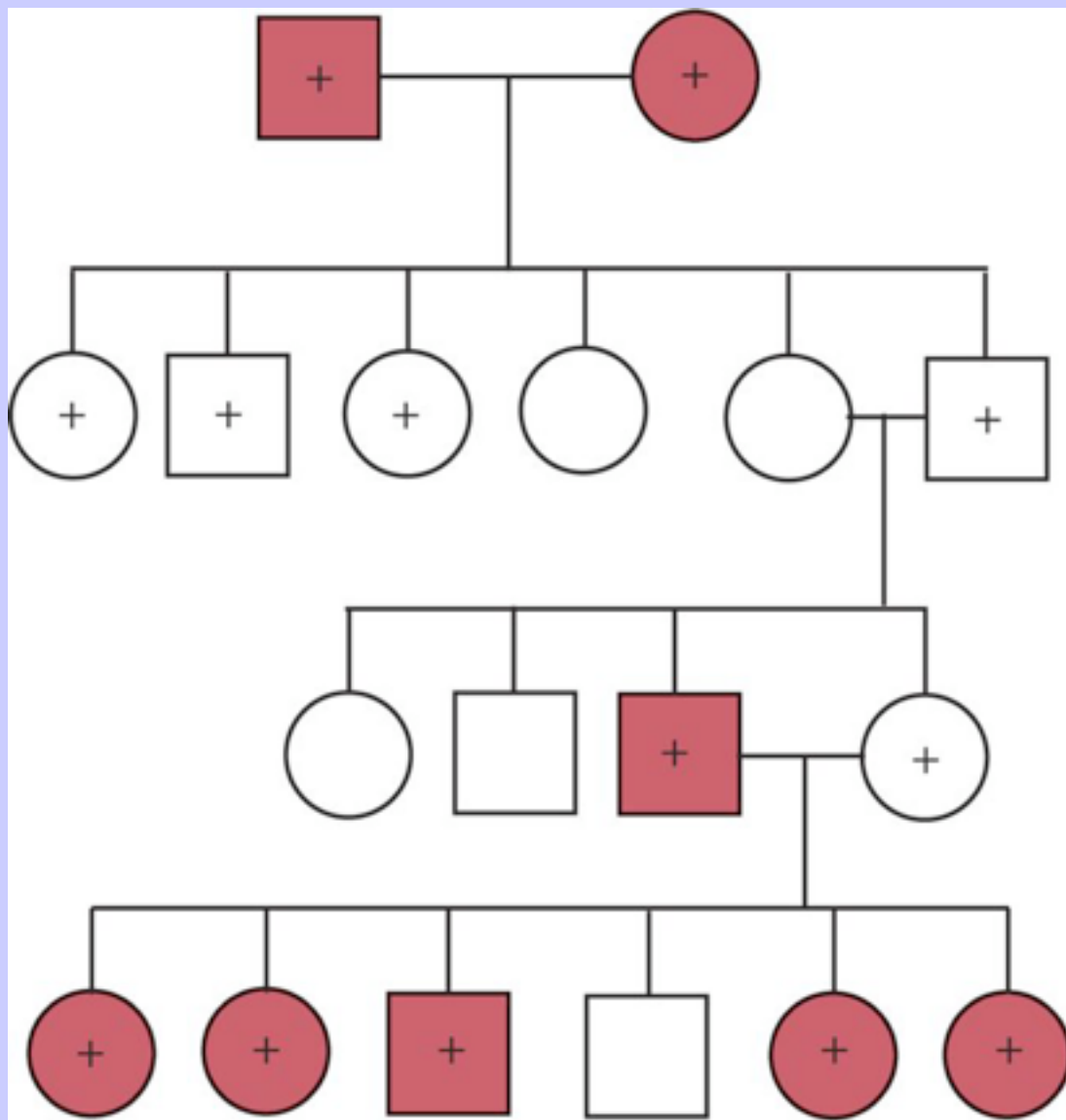
Equine lupus presents as a generalized skin disease (alopecia, dermal ulceration, and crusting) accompanied by an antiglobulin-positive anemia. The disease is remarkable insofar as affected horses may be almost totally hairless ([Figure 33-5](#)). Affected horses are ANA-positive, although LE cell tests are equivocal in this species. Skin biopsies show basement membrane degeneration and immunoglobulin deposition typical of lupus. Affected horses may also have glomerulonephritis, synovitis, and lymphadenopathy. Treatment of reported cases has been unsuccessful.

33.2.1.2 Canine Lupus

Lupus affects middle-aged dogs (between 2 and 12 years of age) and affects males more than females. The disease is commonly seen in Collies, German Shepherds, and Shetland Sheepdogs, but Beagles, Irish Setters, Poodles, and Afghan Hounds are also affected. Lupus (or positive lupus serology) may occur in re-lated animals, supporting the importance of genetic factors. For example, dogs possessing the MHC class I dog leukocyte antigen (DLA)-A7 are at increased risk and those possessing DLA-A1 and B5 are at decreased risk for developing disease ([Figure 33-6](#)). When dogs affected by lupus are bred, the number of affected offspring is higher than can be accounted for genetically, suggesting that the disease may be vertically transmitted. Cell-free filtrates from asymptomatic but LE cell-positive dogs, when administered to newborn mice, have been reported to provoke the appearance of ANA and the development of some lymphoid tumors. Type C retroviruses have been isolated from these tumors, and antisera to these viruses may be used to demonstrate viral antigens on the lymphocytes and in the glomeruli of humans with lupus. Cell-free filtrates of these mouse tumors have also been reported to

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FIGURE 33-6 The inheritance of canine systemic lupus erythematosus. This diagram shows four generations of a single family of dogs. Colored squares (male) or circles (female) denote those animals exhibiting clinical signs of systemic lupus; “+” denotes animals positive for antinuclear antibodies. (From Teichner M, Krumbacher K, Doxiadis I, et al: *Clin Immunol Immunopathol* 55:225, 1990.)



induce the formation of ANA and the production of LE cells in newborn puppies.

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Dogs may present with one or more signs of disease. However, the disease is progressive, so the severity of the lesions and the number of organ systems involved gradually increases in untreated cases. The most characteristic presentation is a fever accompanied by a symmetrical, nonerosive polyarthritis. Indeed, as many as 90% of dogs with lupus may develop arthritis at some stage. Other common presenting signs include renal failure (65%), skin disease (60%), lymphadenopathy and/or splenomegaly (50%), leukopenia (20%), hemolytic anemia (13%), and thrombocytopenia (4%). Dogs may also show myositis (8%) or pericarditis (8%) and neurological abnormalities (1.6%). The leukopenia involves a major loss of CD8⁺ T cells with a somewhat smaller loss of CD4⁺ T cells so that the CD4/CD8 ratio may climb as high as 6, compared with a normal value of about 1.7. The skin lesions are highly variable but are commonly restricted to areas exposed to sunlight. With this great variety of clinical presentations to choose from, it is not surprising that lupus is so difficult to diagnose.

Several unique variants of lupus have been de-scribed in dogs. All are very rare. Many are associated with specific breeds, strongly suggesting a genetic predisposition.

Vesicular systemic lupus is a variant of lupus seen in Shetland Sheepdogs and Rough-Coated Collies. It is characterized by vesicular erosive and ulcerative skin lesions, subepidermal vesicles, and immunoglobulin deposition binding to keratinocyte cytoplasm and/or the dermal-epidermal junction. Affected animals have antibodies against type VII collagen as well as ANAs. It may be treated with aggressive immunosuppressive therapy.

Exfoliative lupus dermatitis has been described in German Short-Haired Pointers. Young adult dogs develop scaling and alopecia on the muzzle, pinnae, and dorsum. Some dogs may exhibit signs of pain and arthritis. Others may develop anemia and thrombocytopenia. Histopathology shows hyperkeratosis with a lymphocytic interface dermatitis similar to that seen in human lupus. IgG is deposited in the epidermal and follicular basement membranes. These dogs have circulating autoantibodies to epidermal basement membranes. Affected animals respond poorly to immunosuppressive therapy.

Another unique lupus-related disease has been described in Gordon Setters. These dogs have developed symmetrical onychodystrophy—malformations and loss of the claws. As a result, affected animals show lameness, severe discomfort, and acute pain. Some develop ANAs. A related disease of Gordon Setters is possibly “black hair follicular dysplasia.” In this disease, dogs begin to shed their black hair without normal regrowth occurring. The remaining black hair is either short and stiff or is thin and easily removed. Many affected dogs have positive ANA titers. These two diseases, occurring in the same breed and often in the same individual, may be closely related.

33.2.1.3

Feline Lupus

Lupus is uncommon in cats, in which it usually presents as an antiglobulin-positive anemia. Other clinical manifestations include fever, skin disease, thrombocytopenia, polyarthritis, and renal failure. The ANA test must be interpreted with care in cats since many normal cats are ANA-positive.

33.2.2

Diagnosis

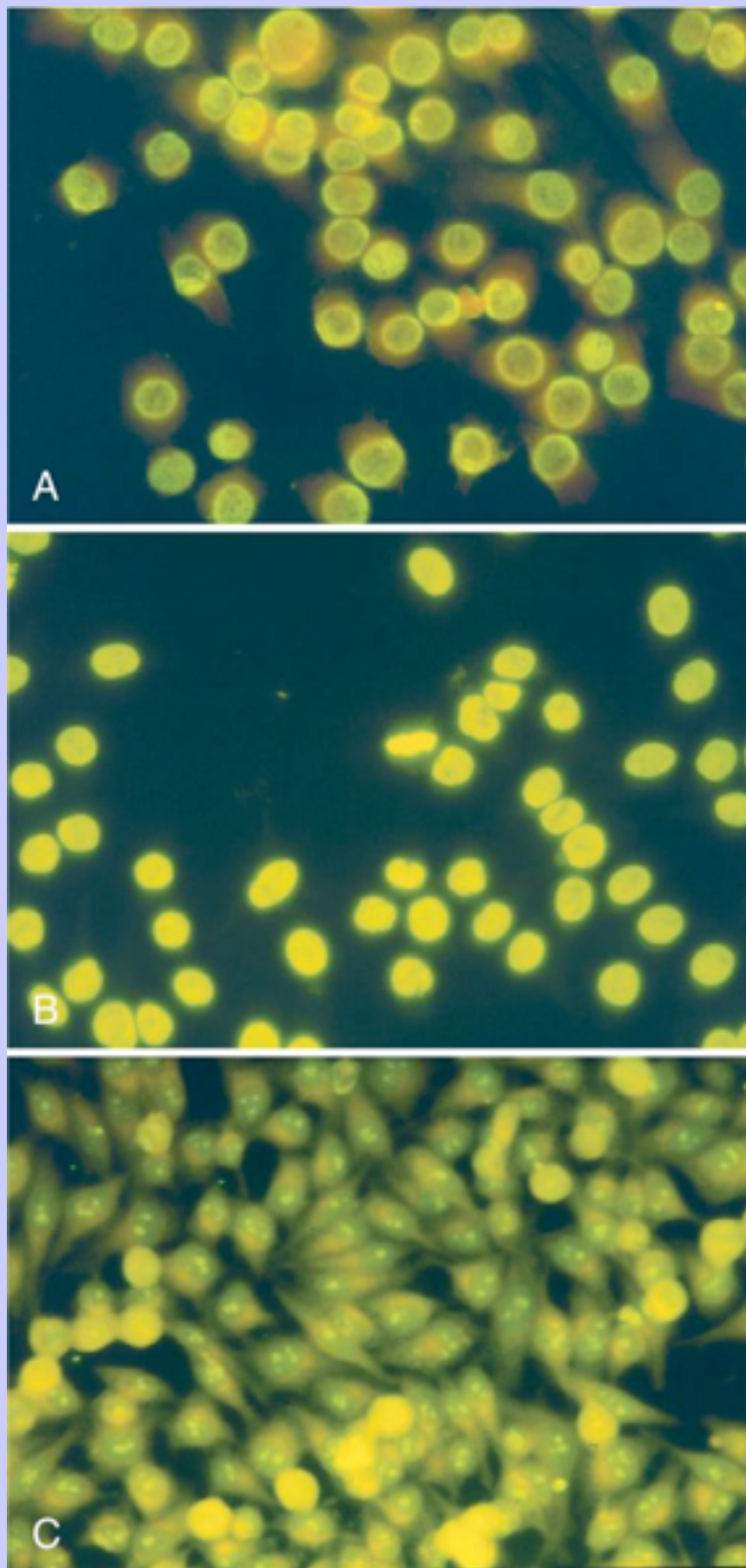
A simple diagnostic rule for lupus could be stated as follows: Suspect lupus in an animal with multiple disorders such as those described above and either a positive test for ANA or a positive test for LE cells ([Box 33-1](#)).

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ANAs are normally demonstrated by immunofluorescence. Cultured cells or frozen sections of mouse or rat liver on a microscope slide are used as a source of antigen. Dilutions of a patient's serum are applied to this, and the slide is incubated and then washed off.

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FIGURE 33-7 Three positive antinuclear antibody reactions. These are indirect fluorescent antibody reactions, in which dog serum under test is layered onto a cell culture. After washing, the bound antibody is detected using a fluorescent antiglobulin. Although “rim” fluorescence **(A)** has traditionally been considered a positive reaction, the staining pattern obtained appears to depend in large part on the way the cells are fixed. These can therefore show diffuse staining **(B)** or nucleolar fluorescence **(C)**. (Courtesy Dr. F.C. Heck.)



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Binding of ANA to the cell nuclei is revealed by incubating the tissue in a fluorescein-labeled antiserum to canine or feline immunoglobulins and then rewashing. A variety of different nuclear staining patterns have been described for humans and their clinical correlations identified. In animals, staining patterns have been less thoroughly investigated, and their significance is unclear. Evidence suggests that a homogeneous staining pattern or staining of the nuclear rim is of greatest diagnostic significance but that nucleolar fluorescence is not ([Figure 33-7](#)). Dogs whose serum shows a speckled fluorescence pattern tend to have autoimmune diseases other than lupus. Some normal dogs, dogs undergoing treatment with certain drugs (griseofulvin, penicillin, sulfonamides, tetracyclines, phenytoin, procainamide), and some dogs with liver disease or lymphosarcoma may have detectable ANAs. ANAs are found in a significant proportion of dogs infected with *Bartonella vinsonii* ssp. *Berkhoffi*, *Ehrlichia canis*, and *Leishmania infantum*. Dogs infected with multiple vector-borne organisms are especially likely to be ANA positive.

33.2.2.1

Box 33-1 Diagnostic Criteria for Systemic Lupus Erythematosus

Any two of the following must be present:

- Characteristic skin lesions
- Polyarthritis
- Antiglobulin-positive hemolytic anemia
- Thrombocytopenia
- Proteinuria

And either

- A positive antinuclear antibody test

Or

- A positive lupus erythematosus cell test

Thus nonspecific ANAs may be a result of many different neoplastic, inflammatory, and autoimmune diseases. ANA test results must therefore be used with caution. Administration of propylthiouracil to cats with hyperthyroidism may result in the development of a syndrome resembling lupus. This may include the development of an antiglobulin-positive anemia, as well as positive ANA reactions.

LE cells, as previously mentioned, are neutrophils (polymorphonuclear neutrophils [PMNs]) that have phagocytosed nuclei from dead and dying cells (see [Figure 33-3](#)). Their presence may be detected in the bone marrow and occasionally in buffy coat preparations from animals with lupus. It is usually necessary, however, to produce them in vitro. This can be accomplished by allowing the blood of an affected animal to clot and then incubating it at 37° C for 2 hours. During this time, normal PMNs will phagocytose the nuclei of any dying or damaged cells. Pressing it through a fine mesh then disrupts the clot, the resulting cell suspension is centrifuged, and the buffy coat is smeared, stained, and examined. LE cells are not a reliable diagnostic feature of systemic lupus in domestic animals since there is a high incidence of both false-positive and false-negative results.

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33.2.3 Treatment

Lupus in animals usually responds well to high doses of corticosteroids (prednisolone or prednisone) accompanied, if necessary, by cyclophosphamide, azathioprine, or chlorambucil. Levamisole (see [Chapter 36](#)) has also been used with success. However, more drastic measures, such as plasmapheresis, may be needed in refractory cases.

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33.3 DISCOID LUPUS ERYTHEMATOSUS

Discoid lupus erythematosus is a mild, uncommon variant of SLE characterized by the occurrence of facial skin lesions alone. There are no other pathological lesions, and ANA and LE tests are negative. It occurs in dogs, cats, horses, and humans. Discoid lupus has been described in Collies and Collie crosses, German Shepherds, Siberian Huskies, and Shetland Sheepdogs. They commonly present with nasal dermatitis with depigmentation, erythema, erosion, ulceration, scaling, and crusting. A vesicular form of the disease has been reported in Shetland sheepdogs. Occasionally the feet may be affected, and some dogs may have oral ulcers. C3, IgA, IgG, or IgM may be detected in the skin basement membrane in a typical lupus band. The skin lesions may be infiltrated with mononuclear and plasma cells. It is treated with corticosteroids, and the prognosis is good. Since the lesions are exacerbated by sunlight, it is appropriate to use sunscreens and encourage the owner to keep the animal out of intense sunlight.

Discoid lupus in cats is characterized by a nonpruritic scaling and crusting dermatitis almost totally confined to the pinnae of the ear. There may be some ulceration and papule or pustule formation. Skin biopsy shows mononuclear infiltration of the basal cell layer with degeneration of basal cells. Direct immunofluorescence of skin sections shows a lupus band. Affected cats have negative or low ANA titers and negative LE cell tests. Treatment with corticosteroids is effective.

33.4 SJÖGREN'S SYNDROME

The triad of keratoconjunctivitis sicca, xerostomia, and rheumatoid factor (RF) constitute an autoimmune syndrome called Sjögren's syndrome. In this syndrome, autoimmune attack on salivary and lacrimal glands leads to conjunctival dryness (keratoconjunctivitis sicca) and mouth dryness (xerostomia). Affected animals subsequently develop gingivitis, dental caries, and excessive thirst. Sjögren's syndrome is often associated with rheumatoid arthritis, systemic lupus, polymyositis, and autoimmune thyroiditis. The first two cases described in dogs were found in a colony maintained for investigations into canine lupus. Affected dogs develop antibodies to nictitating membrane epithelial cells and, less consistently, to lacrimal and salivary glands or to the pancreas, and these organs may be extensively infiltrated with lymphocytes and other mononuclear cells. Most affected animals (90%) are hypergammaglobulinemic and have ANAs (40%) and RFs (34%). Many have other autoimmune lesions such as polyarthritis, hypothyroidism, or glomerulonephritis.

33.4.1 Keratoconjunctivitis Sicca

In keratoconjunctivitis sicca, one of the most common ophthalmic diseases of dogs, lacrimal gland secretion is greatly reduced and animals experience corneal dryness. The resulting abrasion leads to inflammation of the cornea and conjunctiva. There is a mucoid or mucopurulent ocular discharge, as well as blepharitis, conjunctivitis, and secondary bacterial infections. Corneal ulceration may occur; this can progress to perforation if untreated.

The disease is diagnosed by use of the Schirmer tear test. A 5- × 30-mm strip of filter paper is placed in the medioventral cul-de-sac for 1 minute. Normal dog tears wet between 14 and 24 µm of paper/min, but in keratoconjunctivitis sicca cases the tears usually wet less than 10 µm and many wet less than 5 µm. The breeds at highest relative risk include English Bulldogs, West Highland White Terriers, Lhasa Apsos, Pugs, Cocker Spaniels, and Pekingese. The disease may be treated by use of artificial tears. It is also logical to use immunosuppressive agents in refractory cases. For example, cyclosporine ophthalmic drops appear to be effective, although it takes 2 to 3 weeks before improved lacrimation is seen.

Keratoconjunctivitis sicca has been reported in a horse. The 3-year-old animal presented with bilateral ulcerative keratoconjunctivitis sicca and improved clinically with ophthalmic cyclosporine therapy. Histological examination of the lacrimal glands showed an eosinophil infiltration with lesser numbers of lymphocytes, plasma cells, and macrophages. Although this suggests an immunological origin for the disease, it must be pointed out that nonimmunological mechanisms such as facial nerve damage can also result in corneal dryness.

33.4.2 Chronic Superficial Keratitis

Chronic superficial keratitis is a common ocular disease of dogs in which blood vessels, lymphocytes, plasma cells, and melanocytes invade the superficial corneal stroma. Immunoglobulin deposits may be present. Eventually the pigmented granulation tissue causes corneal opacity. The disease is believed to be immune mediated, although its cause is unknown. It is more prevalent in dogs living at high altitudes, where UV exposure is great.

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33.5 AUTOIMMUNE POLYARTHRITIS

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Animals can develop immunologically mediated joint diseases. Most are associated with the deposition of immunoglobulins or immune complexes within joints. They may be classified into two groups based on the presence or absence of joint erosion.

33.5.1 Erosive Polyarthritis

33.5.1.1 Rheumatoid Arthritis

The most important immune-mediated erosive polyarthritis in humans is rheumatoid arthritis. Rheumatoid arthritis is a common, crippling disease affecting about 1% of the human population. A very similar disease is seen in domestic animals, especially dogs, in which there is no obvious breed or sex predilection. Dogs with rheumatoid arthritis may present with chronic depression, anorexia, and pyrexia in addition to lameness, which tends to be most severe after rest (for example, immediately after waking in the morning). The disease mainly affects peripheral joints, which show symmetrical swelling and stiffness. Rheumatoid arthritis tends to be progressive and eventually leads to severe joint erosion and deformities. In advanced cases affected joints may fuse as a result of the formation of bony ankyloses. Radiological findings are variable, but the swelling usually involves soft tissues only, and there may be subchondral rarefaction, cartilage erosion, and narrowing of the joint space.

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33.5.1.1.1

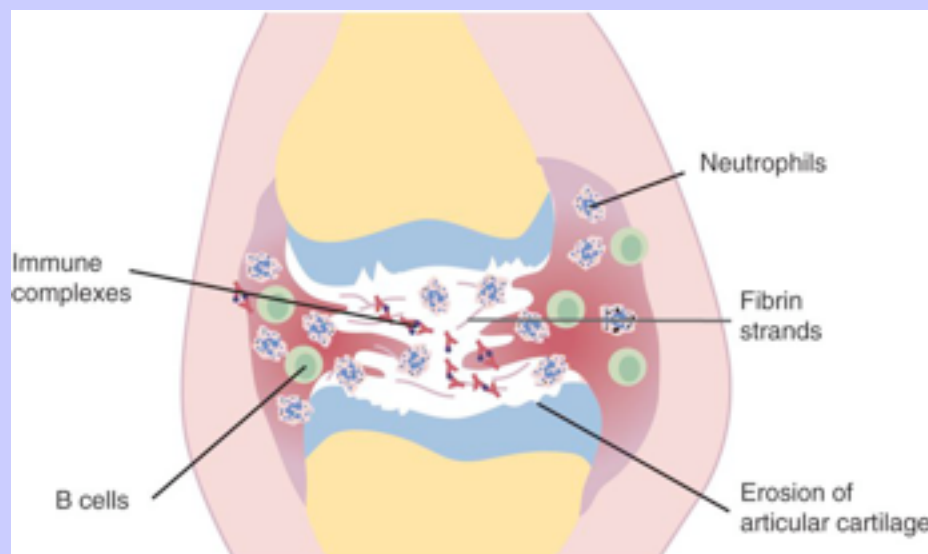
Pathogenesis

Rheumatoid arthritis is a chronic inflammatory disease. It commences as a synovitis with lymphocytes in the synovia and neutrophils in the joint fluid. As the inflammation continues, the synovia swell and proliferate. Outgrowths of the proliferating synovia eventually extend into the joint cavities, where they are called pannus. Pannus consists of fibrous vascular tissue that, as it invades the joint cavity, releases proteases that erode the articular cartilage and, ultimately, the neighboring bony structures. As the arthritis progresses, the infiltrating lymphocytes can form lymphoid nodules and germinal centers within the synovia. Amyloidosis, arteritis, glomerulonephritis, and lymphatic hyperplasia are occasional complications of rheumatoid arthritis ([Figure 33-8](#)).

It is probable that many different stimuli, especially infectious agents, trigger rheumatoid arthritis in susceptible animals. Infectious agents implicated in the human disease include Epstein-Barr virus (a herpesvirus), parvoviruses, and mycobacteria. Lyme disease arthritis has many similarities to rheumatoid arthritis. In domestic mammals, *Mycoplasma hyorhinis*, *Erysipelothrix rhusiopathiae*, and *Borrelia burgdorferi* each produce a chronic arthritis that resembles rheumatoid arthritis. Dogs with rheumatoid arthritis have antibodies to canine distemper in their synovial fluids, antibodies that are not present in dogs with osteoarthritis. Immune complexes can be precipitated out of the synovial fluid of dogs with rheumatoid arthritis, and analysis of these complexes by Western blotting showed the presence of canine distemper virus antigens. Thus canine distemper virus may be present in canine rheumatoid joints and may play a role in the pathogenesis of the disease.

Rheumatoid arthritis is also a genetic disorder. Susceptibility and severity of rheumatoid arthritis in humans are mainly associated with possession of certain MHC class II molecules (human leukocyte antigen [HLA]-DR). This susceptibility is associated with the presence of a conserved amino acid sequence located in the HLA-DRB1 antigen-binding groove and

FIGURE 33-8 A schematic diagram showing how joints are damaged in rheumatoid arthritis.



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known as the “RA shared epitope.” Presumably these molecules can bind and present self-peptides. It is interesting to note that this same conserved RA shared epitope is found on canine DLA-DRB1 and is also associated with susceptibility to rheumatoid arthritis in some dog breeds. Some MHC class III genes also affect susceptibility to canine rheumatoid arthritis. For example, there is an association between possession of the C4 allotype C4-4, low serum C4 levels, and the development of autoimmune polyarthritis. Despite these examples, it has been estimated that non-MHC genes contribute as much as 75% of the genetic susceptibility to rheumatoid arthritis.

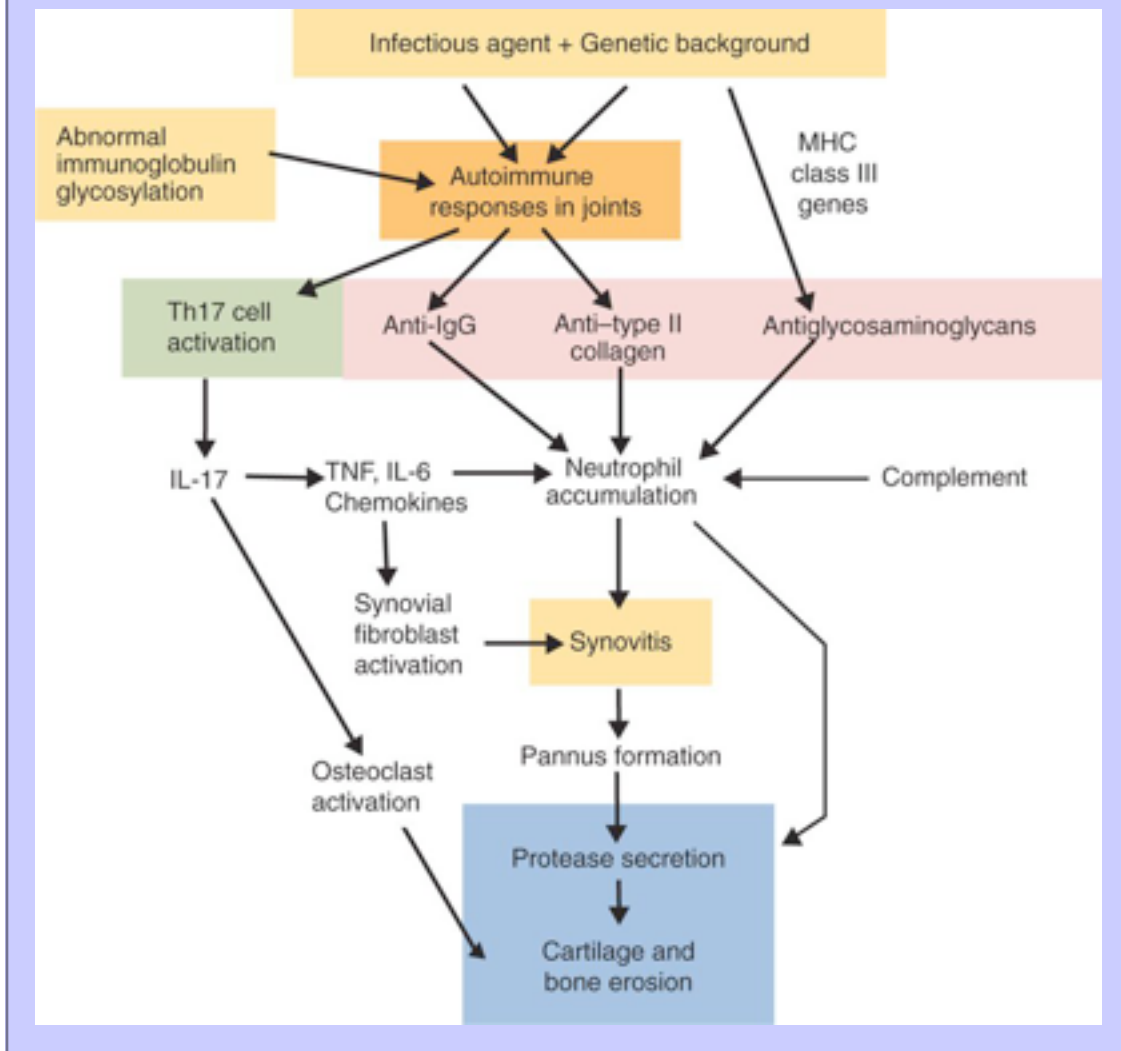
Although rheumatoid arthritis is generally regarded as an autoimmune disease, the identity of the autoantigens involved is unclear. Three major autoantigens that have been implicated are IgG, collagen, and glycosaminoglycans ([Figure 33-9](#)). The development of autoantibodies to IgG is characteristic of rheumatoid arthritis. These autoantibodies, called rheumatoid factors, are directed against epitopes on the C_H2 domains of antigen-bound IgG. They can belong to any immunoglobulin class, including IgE, although IgG RFs are by far the most common. The IgG in rheumatoid arthritis patients is less glycosylated than normal IgG, and it may be that this abnormal IgG can act as an immunogen in a susceptible animal. RFs are found not only in rheumatoid arthritis but also in lupus and other diseases in which extensive immune complex formation occurs. RFs are also found in the serum and synovial fluid of some dogs with osteoarthritis (including cruciate disease) or infective arthritis.

RFs can be detected by allowing them to agglutinate antibody-coated particles. In humans, latex beads coated with IgG are used for this purpose. In dogs, it is easier to make a canine antish sheep erythrocyte serum and coat sheep erythrocytes with this in a subagglutinating dose. After washing, these erythrocytes agglutinate when mixed with RF-positive dog serum.

Although RFs are of diagnostic importance, their clinical significance is unclear. RFs are found in joint fluid, where their titer tends to correlate with the severity of the lesions, and the lesions themselves may be exacerbated by intraarticular inoculation of autologous immunoglobulins. Nevertheless, some individuals with rheumatoid arthritis may not have detectable RFs, and it is not uncommon to find others who have no arthritis despite the presence of RF in their serum. Thus the measurement of RF in dogs is of doubtful specificity.

Other evidence suggests that autoantibodies to collagen may be important. Type II collagen is the

FIGURE 33-9 A schematic diagram showing the possible pathogenesis of rheumatoid arthritis.



predominant form of collagen in articular cartilage and so may serve as an autoantigen. Autoantibodies to type II collagen can be detected in the serum and synovial fluid of dogs with rheumatoid arthritis, infective arthritis, and osteoarthritis. Affected humans develop a cell-mediated response to denatured collagen II and III, and horses with chronic, nonsuppurative arthritis, osteoarthritis, or traumatic arthritis develop antibodies to horse collagens I and II. These antibodies, as well as immune complexes, can therefore be found in the synovial fluid of horses with many different joint diseases. An autoimmune disease that closely resembles rheumatoid arthritis develops in rats immunized with type II collagen. Evidence from experimental mice and some humans suggests that T cells directed against hyaluronic acid, heparin, and chondroitin sulfates may induce an arthritis resembling rheumatoid arthritis.

Whatever the precise initiating factors, the first stage in the development of rheumatoid arthritis probably involves activation of Th17 cells within the synovial membrane. These Th17 cells synthesize IL-17. The presence of IL-17 causes synovial fibroblasts to be activated, and cytokines such as IL-1, IL-6, IL-22,

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granulocyte-macrophage colony-stimulating factor, and tumor necrosis factor- α (TNF- α) are produced by stromal and endothelial cells. Levels of IFN- γ and IL-2 are very low in synovial fluid, suggesting that Th1 cells are not important in this disease. Inflammatory chemokines such as CCL2 (monocyte chemotactic protein-1), CCL3 (macrophage inflammatory protein-1a), and CXCL8 (IL-8) also accumulate. The production of IL-17 together with multiple chemokines, C5a, leukotriene B₄, and platelet-activating factor results in the accumulation of large numbers of neutrophils within the synovial fluid. Activation by phagocytosis of immune complexes and tissue debris leads to protease escape and the release of oxidants. IL-1 and TNF- α stimulate cartilage degradation by activating chondrocytes and stimulating the release of metalloproteases. Thus metalloproteases-2 and -9 from chondrocytes and macrophages are raised in joint fluid from canine cases of rheumatoid arthritis. These metalloproteases can degrade the articular cartilage and ligaments. More importantly, IL-17, TNF- α , and high mobility group box protein-1 produced by activated T cells are potent activators of bone-destroying osteoclasts. Collectively these reactions lead to bone and cartilage erosion and the characteristic joint pathology.

Macrophage cytokines also trigger formation of new blood vessels within synovia. Circulating lymphocytes home to these newly formed capillaries, emigrate into the tissues, and aggregate around the blood vessels. These infiltrating lymphocytes are primarily activated CD4⁺ T cells. B cell emigration into the tissues eventually leads to local RF production. The RFs form large immune complexes and activate the complement system. Some of the immune complexes may precipitate out within the superficial layers of the articular cartilage.

The progressive development of inflammation within the joint leads first to morning stiffness. The joints become warm as the blood flow increases, but because the inflammation is restricted to the synovia, the skin rarely becomes red. The animal may show depression and fatigue as a result of the systemic effects of IL-1 and TNF- α . If the joints develop effusions, they will be obviously swollen. As the disease progresses, the grossly inflamed synovia invades the cartilage, ligaments, and bone and results in the destruction of articular cartilage. Synovial lining cells, small blood vessels, and fibroblasts proliferate. Large numbers of macrophages are found in the pannus, as well as MHC class II-positive nonphagocytic cells—probably B cells and dendritic cells.

Some immunologists believe that rheumatoid arthritis may result from immune responses against citrullinated antigens. Citrulline is an amino acid derived from arginine. It is believed that this conversion plays a role in the preparation of intracellular proteins for apoptosis. Citrullinated antigens are expressed in inflamed joints. Patients develop high levels of autoantibodies to citrullinated antigens well before rheumatoid arthritis lesions develop, and these autoantibodies appear to be highly specific for this disease. They are rarely found in healthy people or in other diseases. It is possible therefore that the key initial lesion in this disease involves autoimmunity to these modified proteins.

33.5.1.1.2

Diagnosis

Diagnosis of rheumatoid arthritis in animals is generally based on the criteria established for human rheumatoid arthritis. They are listed in [Box 33-2](#). Most should have been present for at least 6 weeks. In addition, steps should be taken to exclude systemic lupus (by testing for ANA) and to exclude any infectious cause for the arthritis.

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33.5.1.1.2.1

Box 33-2 Diagnostic Criteria for Canine Rheumatoid Arthritis

- Stiffness or joint pain, especially after periods of inactivity

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- Symmetrical joint swelling, especially if multiple joints are involved
- Sterile synovial fluid containing inflammatory cells
- Positive rheumatoid factor test
- Erosive polyarthritis with characteristic histology

33.5.1.1.3

Treatment

Treatment of canine rheumatoid arthritis with drugs tends to be unsatisfactory, and the long-term prognosis of the disease is poor. Nonsteroidal antiinflammatory drugs, such as aspirin, carprofen, or etodolac, have been the first choice in treating early, uncomplicated cases of rheumatoid arthritis, although their efficacy is unclear. Corticosteroids such as prednisolone should be reserved for late, severe cases in which salicylates have proved inadequate. Local steroid injections into affected joints will produce rapid relief and clinical remission. However, the joints are still subjected to stress, disease progression is not slowed, and the corticosteroids delay healing and promote articular degeneration. Their use may therefore permit articular damage to proceed unabated. Recently, good success has been achieved in humans by the aggressive use of the immunosuppressive agent methotrexate. Monoclonal antibodies to TNF- α (infliximab), to CD4, to thymocytes, or to IL-2R have also had significant success in preventing bone erosions in humans, as has administration of recombinant TNF- α receptors (etanercept). The immunosuppressive drug leflunomide appears to be as effective as methotrexate. Slow-acting immunosuppressive agents, such as sodium aurothiomalate and aurothioglucose, and antimalarials, such as chloroquine, are also used in humans, but they are expensive, results have been erratic, and experience with these in animals is limited. Appropriate surgery may improve joint stability and reduce pain.

33.5.2

Nonerosive Polyarthritis

The second major group of immune-mediated arthritides are those in which the joint cartilage is not eroded and the inflammatory lesion is largely confined to the joint capsule and synovia. Many of these clinically resemble rheumatoid arthritis but may be differentiated by their nonerosive character. Nonerosive polyarthritis is classified as type 1 disease when an animal suffers from polyarthritis alone; type 2 disease is associated with infections present in other body systems; type 3 disease is associated with gastrointestinal disease; and type 4 disease is associated with the presence of a neoplasm elsewhere in the body.

33.5.2.1

Equine Polyarthritis/Polysynovitis

Polyarthritis has been reported in foals in association with a lupuslike syndrome. In these cases affected foals (up to 3 months of age) present with multiple swollen joints involving all four limbs and a persistent fever. In some cases other synovial sheaths, including tendon sheaths and bursae, are affected. The synovial effusions are sterile, but synovial biopsies show lymphocyte and plasma cell infiltration with some immunoglobulin deposits. The cells in the joint fluid are mainly neutrophils. These animals are negative for RF, ANA, and LE cells. Many of these animals have a lesion within the thorax, especially *Rhodococcus equi* pneumonia. This is classified as a type 2 disease. It is possible that immune complexes originating in the lungs may lodge in the synovia to trigger the synovitis. The polyarthritis usually resolves as the primary lesion resolves.

A type 1 immune-mediated polyarthritis has also been recorded in horses. In these cases animals lose weight, develop an intermittent fever, and have effusions in multiple joints leading to stiffness. They have systemic signs of inflammation including anemia, leukocytosis, hyperfibrinogenemia, and hyperglobulinemia. The synovial effusion is sterile, and immunoglobulins are present in the synovial membrane. The condition usually resolves on steroid and immunosuppressive therapy.

33.5.2.2

Canine Polyarthritis

Dogs may develop several distinct nonerosive poly-arthritides, which can be divided into at least three major categories: arthritis associated with systemic lupus, arthritis associated with a myositis, and an idiopathic polyarthritis. Breeds that are predisposed to polyarthritis include German Shepherds, Irish Setters, Shetland Sheepdogs, Cocker Spaniels, and Springer Spaniels. The main clinical features of polyarthritis are stiffness, pyrexia, anorexia, and lethargy.

33.5.2.2.1

Lupus Polyarthritis

Polyarthritis is a common feature of systemic lupus. Diagnosis is contingent on making a firm diagnosis of lupus. Thus it is necessary to show multiple system involvement, a significant titer of serum ANAs, and immunopathological features consistent with lupus.

33.5.2.2.2

Polyarthritis with Polymyositis

A disease characterized by both nonerosive polyarthritis and polymyositis is recognized in young dogs. Most recorded cases have been seen in spaniels. The animals are stiff and have painful joints, fever, lethargy, weakness, muscle atrophy, and muscle pain. They are negative for both ANA and RF. The arthritis is nonerosive and symmetrical, involving multiple joints. The animals have a symmetrical inflammatory myopathy with myalgia, atrophy, and muscle con-tracture. The synovial fluid shows high white cell counts, especially neutrophils. Muscle biopsies show a neutrophil or mononuclear cell infiltrate, or both, with muscle fiber atrophy and degeneration. Synovial biopsies show a neutrophil and mononuclear cell infiltration with a fibrinous exudate. IgG, IgM, and complement are deposited in the walls of the synovial vessels. Animals may be treated with corticosteroids and immunosuppressive agents such as cyclophosphamide.

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Table 33-1 Classification of Nonerosive Polyarthritis in Dogs

Type	Disease Associations
I	Uncomplicated polyarthritis without other disease associations
II	Polyarthritis associated with infectious lesions remote from the joints (e.g., respiratory or urinary infections)
III	Polyarthritis associated with gastrointestinal disease
IV	Polyarthritis associated with neoplastic disease remote from the joints
From Bennett DJ: <i>J Small Anim Pract</i> 28:909-928, 1987.	

33.5.2.2.3

Idiopathic Polyarthritis

Most cases of canine polyarthritis fit none of the categories described above. Although these cases are nonerosive and possess the characteristics of type III hypersensitivity, their precise etiology is unknown ([Table 33-1](#)).

An example of type 1 polyarthritis is the juvenile polyarthritis syndrome seen in Akitas between the ages of 9 weeks and 8 months. These dogs have a cyclical high fever lasting 24 to 48 hours before resolving and evidence of severe, incapacitating joint pain with soft tissue swelling. Radiology shows hepatosplenomegaly and lymphadenopathy. Some animals may have meningitis or meningoencephalitis. Their erythrocytes may be antiglobulin-positive. Synovial fluid shows no evidence of infection, although large numbers of neutrophils are present. The dogs are usually negative for RF and ANA. Pedigree analysis suggests that the disease is inherited. Some dogs respond positively to corticosteroid treatment. In refractory cases azathioprine may also be required.

Type 2 disease is a reactive arthritis associated with infections in the respiratory or urinary tract, tooth infections, or cellulitis. Type 3 disease is associated with the presence of gastroenteritis, diarrhea, or ulcerative colitis. It is not clear whether this type of disease is truly distinguishable from type 2 disease. Type 4 disease is associated with the presence of tumors, including seminomas and carcinomas of several types.

Idiopathic polyarthritis tends to be most common in male dogs, and about half of the cases are seen in young dogs between 1 and 3.5 years. Most of the animals show systemic signs such as fever, anorexia, and lethargy. The animals are lame and have a history of stiffness after rest. The most commonly affected joints include the stifle, elbow, and carpus. The onset of lameness is sudden in most cases and is associated with obvious muscle atrophy. There is no significant joint erosion, although periarticular soft tissue swelling and synovial effusion are common. Some cases may have proliferative periosteal changes. All cases are negative for RF and ANA. The joint fluid is sterile. Synovial biopsies show hypertrophy with a neutrophil and/or a mononuclear cell infiltration. Fibrin deposits are seen in most cases, as is fibrosis. Most lesions contain IgM, IgG, and complement deposits, and some contain IgA-producing plasma cells. Some affected dogs may show evidence of glomerulonephritis. Animals respond well to corticosteroids. The prognosis for idiopathic polyarthropathy is generally better than that for the other forms of immune-mediated arthritis.

33.5.2.3

Feline Polyarthritis

Chronic progressive polyarthritis of male cats is characterized by polyarthritis with either osteopenia or periosteal new bone formation. Periarticular erosions and eventual collapse or subchondral erosions, joint instabilities, and deformities closely resembling those of rheumatoid arthritis are also seen. Affected cats are commonly infected with feline syncytia-forming virus (FSV) or feline leukemia virus (FeLV), or both. (The incidence of FSV in these cats is 2 to 4 times higher, and the incidence of FeLV is 6 to 10 times higher than in normal cats.) It is described here because of suggestions that it is of immunological origin. These suggestions are based on the massive lymphocyte and plasma cell infiltration of affected joints and the presence of an immune complex type of glomerulonephritis. However, affected cats are RF- and ANA-negative, and their serum immunoglobulin levels tend to be close to normal. Corticosteroids lessen the severity of clinical signs. Combination therapy with corticosteroids and azathioprine or cyclophosphamide can induce temporary remissions.

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33.5.2.4

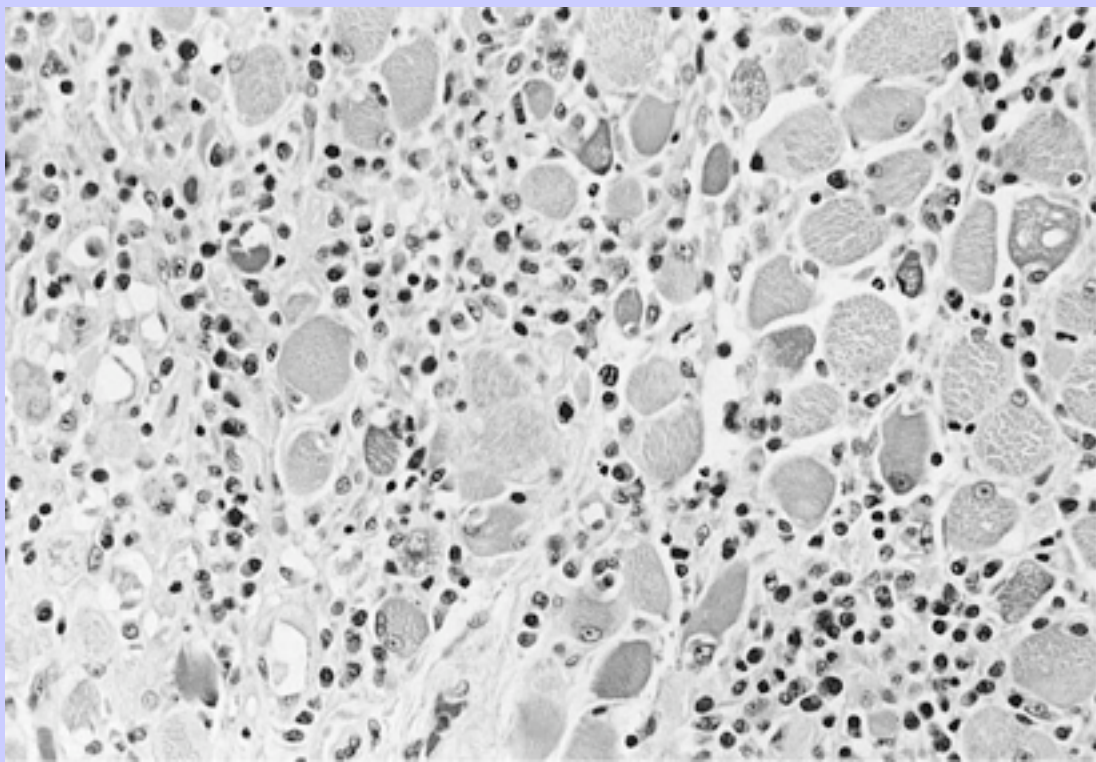
Cruciate Ligament Rupture

Immunological abnormalities are associated with spontaneous rupture of the anterior cruciate ligament in dogs. For example, the synovia of affected dogs contains B cells and IgG-positive plasma cells. In addition the synovia contains numerous MHC class II⁺, CD1c⁺ dendritic cells. Autoantibodies to both type I and type II collagen are found in synovial fluid following cruciate ligament rupture (secondary to osteoarthritis). These antibodies are largely bound in immune complexes. They are unlikely to be of major significance

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FIGURE 33-10 A section of esophagus from a dog with dermatomyositis. Note the fragmented myofibers, as well as the infiltration by lymphocytes, plasma cells, and macrophages (H&E stain, ×200). (From Hargis AM, Prieur DJ, Haupt KH, et al: *Am J Pathol* 123:480-496, 1986.)



and probably represent a secondary response to local damage.

33.6

DERMATOMYOSITIS

A familial disease of dogs that resembles dermatomyositis in humans has been described in Collies and Shetland Sheepdogs. The disease is inherited as an autosomal dominant condition involving a locus on chromosome 35, although expression is highly variable. Dogs develop dermatitis with a less obvious myositis. Puppies appear normal at birth, but skin lesions develop between 7 and 11 weeks of age and myositis develops between 12 and 23

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weeks. In other studies, the dermatitis developed at 3 to 6 months of age, and myositis was detected after the dermatitis was investigated. The dermatitis first develops on the face; subsequently, lesions may spread to the limbs and trunk, especially over bony prominences. These early lesions are erythematous and eventually lead to vesicle and pustule formation. Once the vesicles rupture, they ulcerate and crust. Lesions may be found on the bridge of the nose and around the eyes and show hair loss and changes in pigmentation. There may be enlargement of the lymph nodes draining the affected areas. The clinical course and severity are variable, but skin lesions usually resolve by 1 year of age.

Muscle disease follows the onset of skin disease, but there is no correlation between the severity of the two lesions. The most common sign of myositis is masseter and temporal muscle atrophy. Some severely affected puppies may have difficulty eating as a result of the myositis and so grow poorly. If the muscles of the esophagus are affected, megaesophagus may develop and secondary aspiration pneumonia results. Generalized lymphoid hyperplasia may also develop in these dogs. Many dogs outgrow the disease and are left with moderate hyperpigmentation, some hypo-pigmentation and alopecia, and some atrophy of the muscles of mastication. Other dogs develop a progressive disease with severe dermatitis and myositis. Dogs with progressive disease may also develop signs of immunosuppression, especially pyoderma and septicemia, as well as demodicosis. On necropsy, myositis may be seen in the esophagus and arteritis in the skin, muscle, and bladder.

The onset and progression of the disease are correlated with a rise in circulating immune complexes and serum IgG, but the reason for these increases is unclear. Circulating immune complexes and IgG levels return to normal as the disease resolves, suggesting a causative association. Histology shows a nonspecific inflammatory dermatitis. Muscle biopsy, especially of the temporal muscle, shows multifocal accumulations of lymphocytes, plasma cells, and macrophages, as well as a few neutrophils and eosinophils. The myofibers are atrophied and may show fragmentation and vacuolation ([Figure 33-10](#)). Symptomatic and corticosteroid treatment may be of benefit in severely affected cases.

33.7 IMMUNE VASCULITIS

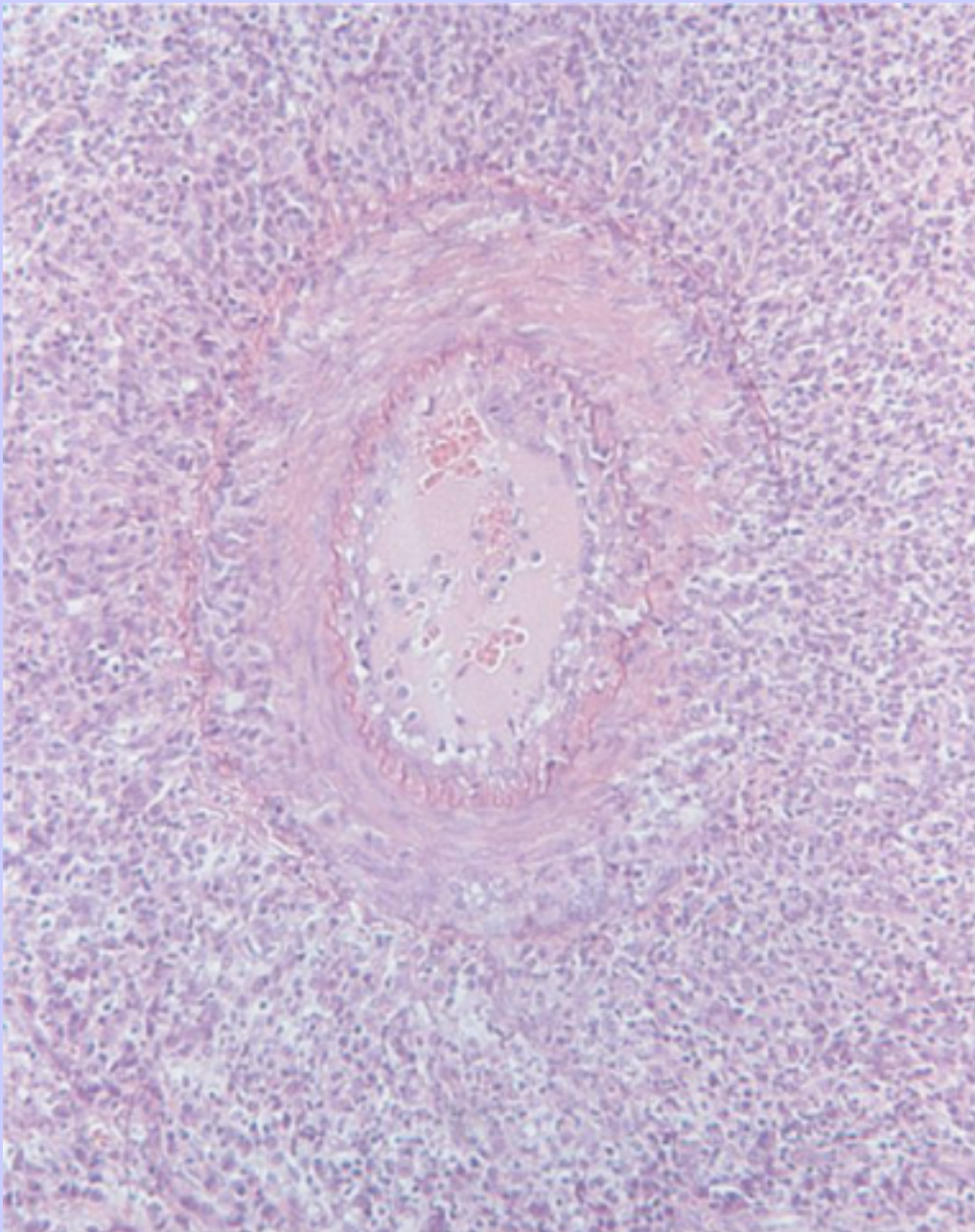
Several forms of immune-mediated vasculitis have been described in domestic animals. Their precise relationships are unclear, and, as a result, they have been given several different names, including canine juvenile polyarteritis, polyarteritis nodosa, and leukocytoclastic vasculitis.

Canine juvenile polyarteritis primarily affects Beagles less than 2 years of age. The animals show episodes of anorexia, persistent fever of greater than 40° C, and a hunched stance with lowered head and a stiff gait, indicating severe neck pain. The clinical signs may show cyclical remissions and relapses. The animals have a neutrophilia and elevated acute-phase proteins. These dogs have elevated serum IgM and IgA but normal IgG. The proportion of B cells in the blood is increased, but their T cells are decreased, as is their response to mitogens.

On necropsy there are few gross lesions. There may be some hemorrhage in lymph nodes. Histologically there is a systemic vasculitis and perivascularitis. In the acute disease there is necrotizing vasculitis with fibrinoid necrosis and a massive inflammatory cell infiltration involving the small- and medium-sized arteries of the heart, mediastinum, and cervical spinal cord ([Figure 33-11](#)). Immunoglobulins are deposited in the walls of small- and medium-sized arteries. During remissions the vascular lesions consist of intimal and

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FIGURE 33-11 An extramural coronary artery from a beagle suffering from juvenile polyarteritis. This medium-sized muscular artery is characterized by medial necrosis, ruptured elastic laminae, and severe perivascular accumulations of neutrophils, lymphocytes, and macrophages. (H&E stain.) (From Snyder PW, Kazacos EA, Scott-Moncrieff JC, et al: *Vet Pathol* 32:337-345, 1995.)



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medial fibrosis and a mild perivascularitis, the residue of previous acute vasculitis. Chronically affected dogs may develop generalized amyloidosis. In many ways, this disease resembles Kawasaki disease of children, the leading cause of acquired heart disease in the United States.

Polyarteritis nodosa occurs in humans, pigs, dogs, and cats. It is characterized by a widespread, focal necrosis of the media of small- and medium-sized muscular arteries. The lesions are found in many organs, especially in the kidney. Vessels in the skin are rarely involved.

On occasion, focal vascular lesions characterized by neutrophil infiltration may develop in small blood vessels throughout the body, but especially in skin. Affected dogs have mucocutaneous ulcers, bullae, edema, polyarthropathy, myopathy, anorexia, intermittent fever, and lethargy. Although called hypersensitivity vasculitis, a foreign antigen can be found in only a small proportion of cases. For this reason, a better name for this condition may be leukocytoclastic vasculitis. The cause or causes of polyarteritis nodosa and hypersensitivity vasculitis are unknown. The histology of both diseases suggests that they are a form of type III hypersensitivity reaction, perhaps due to the presence of an infectious agent. Immunosuppression with corticosteroids, together with cyclophosphamide, has given encouraging results in treating canine hypersensitivity vasculitis. Polyarteritis nodosa is usually detected as an incidental finding on necropsy, although ocular defects may present clinically if the arteries of the eye are involved.

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³⁴ CHAPTER 34 Primary Immunodeficiencies

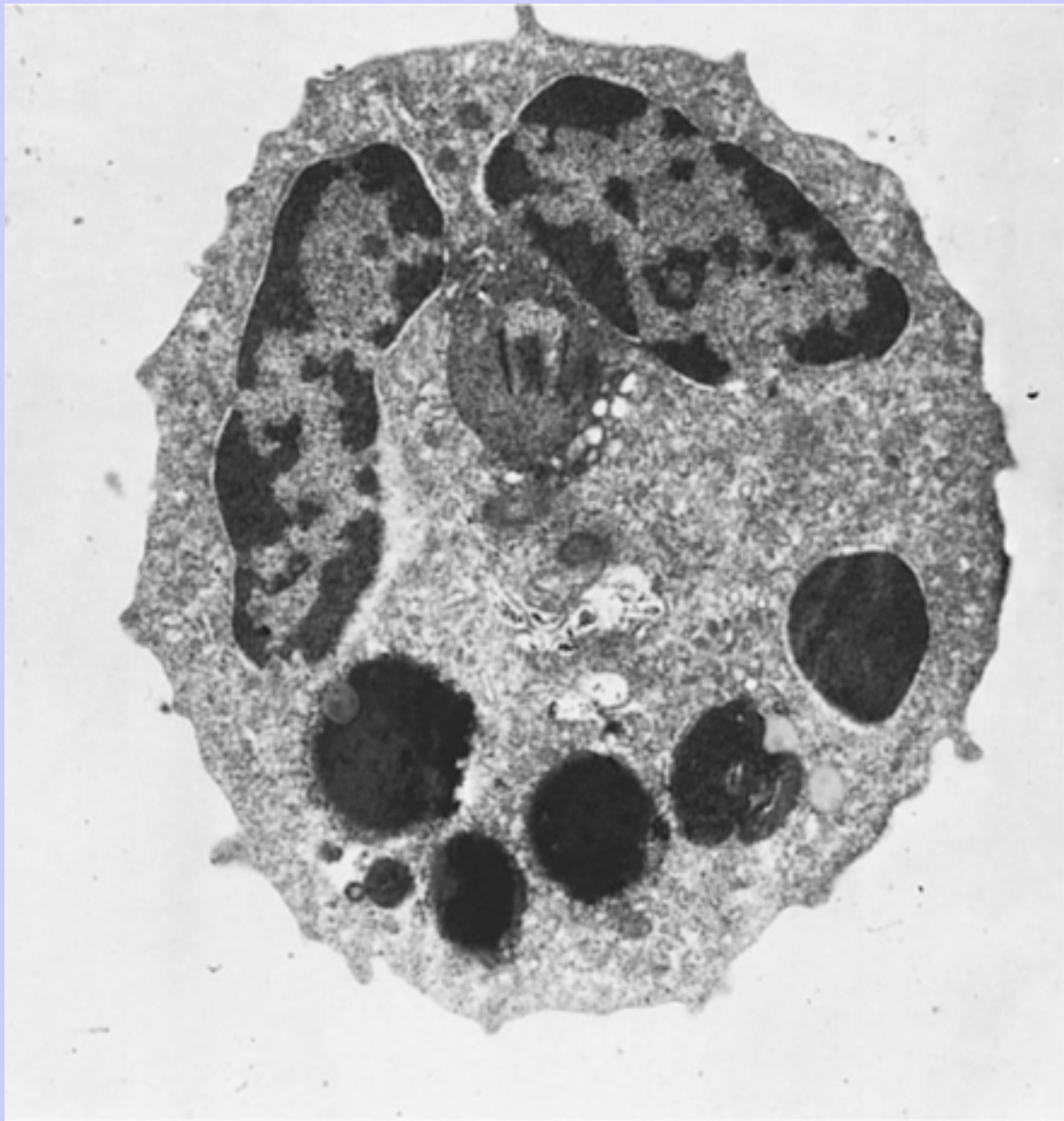
^{34.1} KEY POINTS

- As a result of genetic mutations, defects may develop in the immune system resulting in immunodeficiency in newborn animals.
- Many different defects have now been identified in domestic animals, especially in inbred breeds, where heterozygosity is reduced.
- Defects in innate immunity include deficiencies in phagocytosis and intracellular killing leading to increased susceptibility to bacterial diseases.
- Defects in T cell function generally predispose an animal to overwhelming virus infections.
- Defects in B cell functions predispose animals to overwhelming bacterial disease.
- Combined immunodeficiencies are most severe since affected animals lack resistance to all infectious agents.

Any defect in either the innate or acquired immune systems usually becomes apparent when affected animals show unusual susceptibility to infectious or parasitic diseases. These diseases may be due to pathogenic organisms or, if the defect is very severe, opportunistic infections by organisms that are not normally able to cause disease. Deficiencies in the immune systems may be a result of inherited defects (primary immunodeficiencies), or, alternatively, the deficiencies may be a direct result of some other cause (secondary or acquired immunodeficiencies). This chapter describes some of the primary immunodeficiencies recorded in domestic animals.

One feature of primary immunodeficiencies in domestic animals is breed susceptibility. Examples of breed-associated immunodeficiencies include the increased risk for canine parvoviral enteritis in Doberman Pinschers and Rottweilers. German Shepherd dogs may have increased susceptibility to canine distemper,

FIGURE 34-1 A neutrophil from a Chédiak-Higashi syndrome calf with enlarged cytoplasmic granules. (Courtesy Dr. H.W. Leopold.)



while Mexican Hairless dogs may have defective cell-mediated immune responses. It must also be recognized that the genetic composition of many breeds varies geographically and that problems with a specific breed in one country may not occur in other countries.

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34.2 INHERITED DEFECTS IN INNATE IMMUNITY

Inherited deficiencies in innate immunity include defects in the various stages of phagocytosis, as well as the complement deficiencies described previously (see [Chapter 5](#)). Phagocytic defects are well recognized in domestic animals.

34.2.1 Chédiak-Higashi Syndrome

Chédiak-Higashi syndrome is an inherited disease of Aleutian mink; blue smoke Persian cats; white tigers; Hereford, Japanese black, and Brangus cattle; beige (*bg/bg*) mice; orcas; and humans. It is an autosomal recessive disease resulting from a mutation in a gene (*LYST*) that encodes a protein that controls lysosomal membrane fusion. The *LYST* gene is found on bovine chromosome 28. In Chédiak-Higashi cattle there is a missense A:T → G:C mutation that results in replacement of a histidine with an arginine residue. The defect produces abnormally large secretory lysosomes in neutrophils, monocytes, eosinophils, and pigment cells ([Figure 34-1](#)). The enlarged neutrophil granules result from the fusion of primary and secondary granules. The leukocyte granules of affected animals are more fragile than those of normal animals, rupturing spontaneously and causing tissue damage, such as cataracts in the eye. These leukocytes have defective chemotactic responsiveness, reduced motility, and reduced intracellular killing. Cytotoxic T cells fail to excrete their granzyme-rich lysosomes.

Clinically, the syndrome is associated with multiple abnormalities. Because melanin granules (melanosomes) are related to lysosomes, affected animals show decreased color including dilution of hair pigmentation (sometimes only obvious in the newborn), eye abnormalities, increased susceptibility to infection, and a bleeding tendency. In hair, the melanosomes also fuse causing the dilution of coat color and light-colored irises (pseudoalbinism). Eye abnormalities include photophobia, and animals may develop cataracts. Their eyes have a red fundic light reflection rather than the normal yellow-green.

Because of the neutrophil defects, affected animals may be more susceptible to respiratory infections and neonatal septicemia. Some affected breeds of cattle, such as Herefords, tend to be more susceptible to infection than others, such as Japanese black cattle. The Chédiak-Higashi gene also impairs the function of natural killer (NK) and cytotoxic T cells. As a result, affected animals may show increased susceptibility to tumors and to viruses such as the Aleutian disease virus in mink.

Platelets from affected animals also contain enlarged lysosomes, and their function is abnormal. Affected animals tend to bleed abnormally after surgery and develop hematomas at injection sites. Death from acute hemorrhage is common.

Chédiak-Higashi syndrome may be diagnosed by examining either a stained blood smear for the presence of grossly enlarged granules within leukocytes or by examining hair shafts for enlarged melanosomes. Treatment is symptomatic.

34.2.2 Pelger-Huët Anomaly

Pelger-Huët anomaly is an inherited disorder characterized by a failure of granulocyte nuclei to segment into lobes. As a result, their nuclei remain round in shape. The neutrophils therefore appear on first sight to be very immature (a left shift). The anomaly is usually detected when an animal is observed to have a persistent left shift that cannot be reconciled with its good health. Although Pelger-Huët neutrophils closely resemble band forms,

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their nuclear chromatin is condensed, reflecting their maturity. The disorder in humans is due to a mutation in the gene coding for lamin B, a nuclear membrane receptor that interacts with chromatin to determine the shape of the nucleus. Pelger-Huët anomaly has been observed in humans, domestic shorthair cats, and various dog breeds such as Cocker Spaniels, Basenjis, Boston Terriers, Foxhounds, and Coonhounds. In Foxhounds and Australian Shepherds, the disorder is inherited as an autosomal dominant trait.

Pelger-Huët anomaly has a minimal effect on the health of animals. Nevertheless, fewer pups are weaned from affected dogs than from unaffected ones. In addition, Pelger-Huët neutrophils are less able to emigrate from blood vessels *in vivo*. This reduced mobility may be due to inflexible nuclei. B cell responses may also be impaired in these dogs since normal canine B cells exposed to serum from affected dogs show depressed responses to antigens.

34.2.3

Canine Leukocyte Adhesion Deficiency

In order for neutrophils to leave inflamed blood vessels, they must first bind to vascular endothelial cells. This adherence is mediated by the integrins on the neutrophil surface. In the absence of these integrins, neutrophils cannot bind to endothelial cells and are unable to emigrate into tissues ([Figure 34-2](#)). Thus bacteria in tissues can grow freely without fear of attack by neutrophils. Canine leukocyte adhesion deficiency (CLAD) results from a defect in the integrin CD11b/CD18 (Mac-1). In Mac-1–deficient dogs, neutrophils cannot respond to chemoattractants, bind to complement-coated particles (Mac-1 is a complement receptor), or bind to endothelial cells. Affected dogs have recurrent infections, despite the fact that they have large numbers of neutrophils in their blood.

CLAD has been described in Irish Red Setters (as well as in the related red and white setter breed), in which it is an autosomal recessive disease. Affected animals die early in life as a result of recurrent severe bacterial infections (osteomyelitis, omphalophlebitis, gingivitis), lymphadenopathy, impaired pus formation, delayed wound healing, weight loss, and fever. Animals have a marked leukocytosis ($>200,000/\text{ml}$), primarily a neutrophilia and eosinophilia. Although these granulocytes look normal, functional tests reveal defects in adherence-dependent activities, including impaired adhesion to glass or plastic surfaces or to nylon wool fibers. They cannot ingest C3b-opsonized particles. Normal canine granulocytes aggregate after activation with phorbol myristate acetate, but those of LAD animals do not. Migration in response to chemotactic stimuli is poor. Neither CD11b nor CD18 can be detected by immunofluorescence.

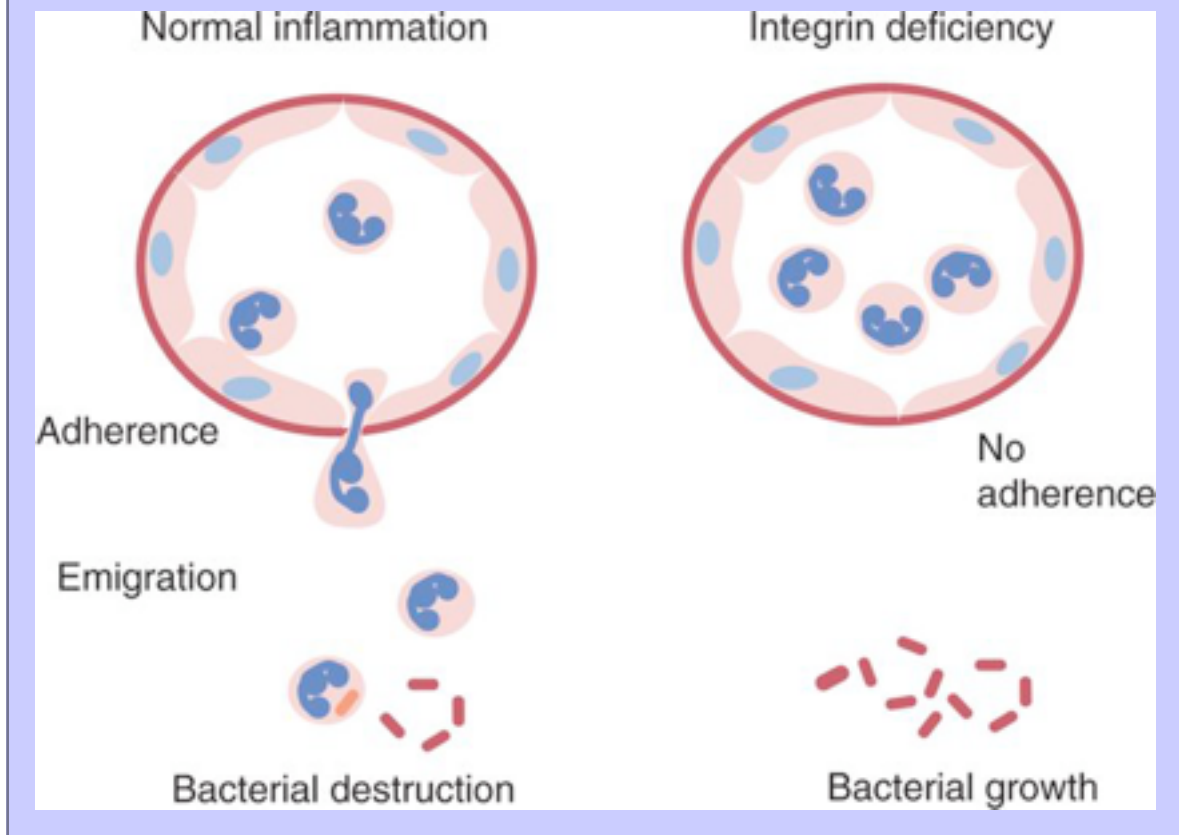
The lesion results from a single missense mutation at position 107 in the β chain of the CD18 gene, which results in the replacement of a highly conserved cysteine residue (Cys36) by a serine. As a result, the mutation disrupts a disulfide bond in CD18, altering its structure and function. CD11b (the α chain) is not expressed, because it must be associated with the β chain before the dimer can be expressed on the cell surface. A diagnostic test for the LAD mutation has been developed. Thus genomic DNA is amplified by polymerase chain reaction (PCR) using primers for the mutated region. The products of this reaction may then be sequenced and the presence of the mutation determined. Matched related bone marrow allografts from normal animals given to LAD dogs have effectively treated the disease.

Canine granulocytopeny syndrome was an autosomal recessive disease observed in Irish setters. Some investigators have suggested that the disease is identical to LAD, but because it was described before integrins were discovered, this cannot be confirmed. These animals had suppurative skin lesions, gingivitis, osteomyelitis, pododermatitis, and lymphadenopathy. Affected dogs had a pronounced leukocytosis, and their neutrophils were morphologically normal, although there was a persistent left shift. The affected animals were hypergammaglobulinemic and anemic as a result of the persistent infections. Their lymph nodes showed diffuse,

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suppurative, nongranulomatous lymphadenitis, which is inconsistent with a diagnosis of LAD. Examination of the neutrophils of these dogs showed that their respiratory burst was depressed, as reflected by a decrease in glucose oxidation. Nevertheless, they were more effective than normal cells at reducing nitroblue tetrazolium, implying that O_2^- was produced in greater quantities than normal or,

FIGURE 34-2 Integrins are required to bind neutrophils firmly to blood vessel walls. This permits the neutrophils to emigrate to sites of bacterial invasion. In the absence of integrins, neutrophil emigration fails to occur. As a result, invading bacteria can grow unmolested in the tissues.



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FIGURE 34-3 The bovine leukocyte adhesion deficiency (*BLAD*) mutation. The mutation involves replacement of a cytosine by a guanosine in the CD18 gene. As a result, an aspartic acid residue is replaced by a glycine residue. The mutation occurs in a highly conserved region of the CD18 molecule and prevents formation of a biologically active molecule.

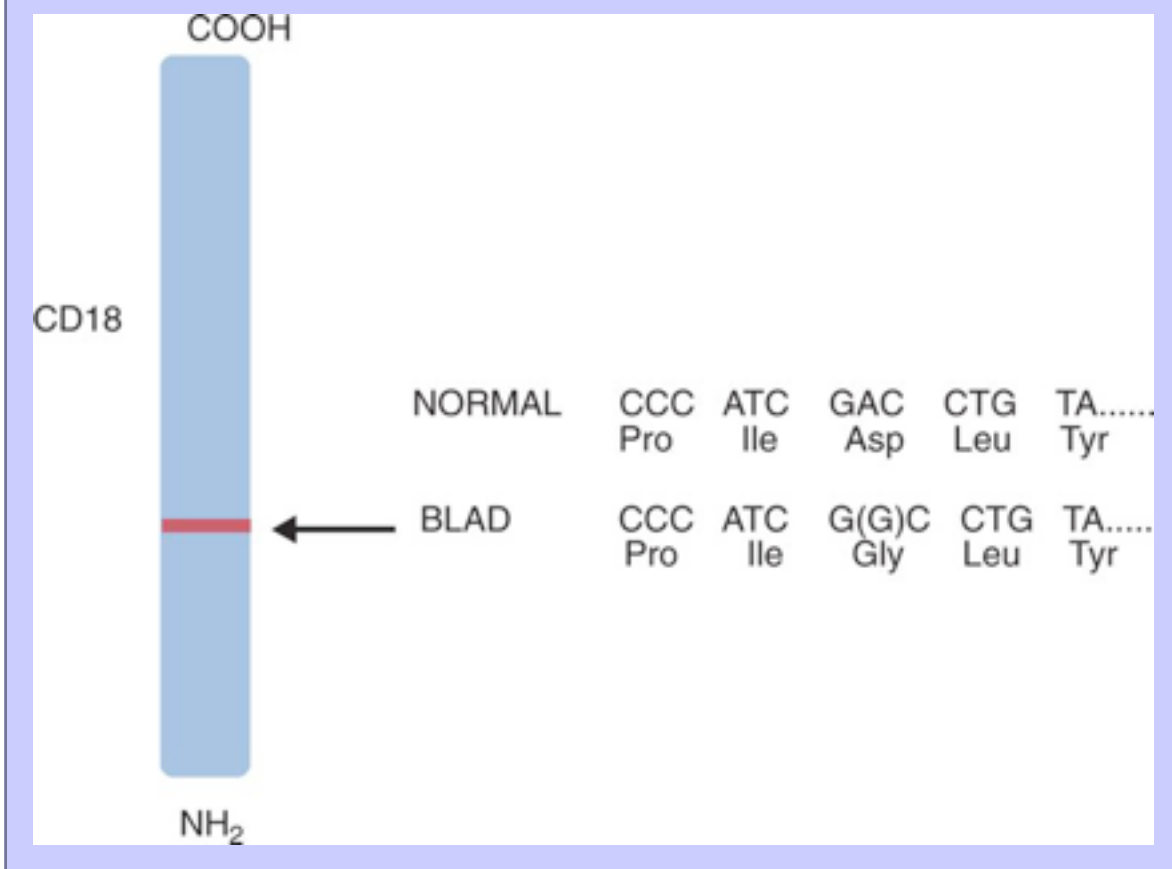
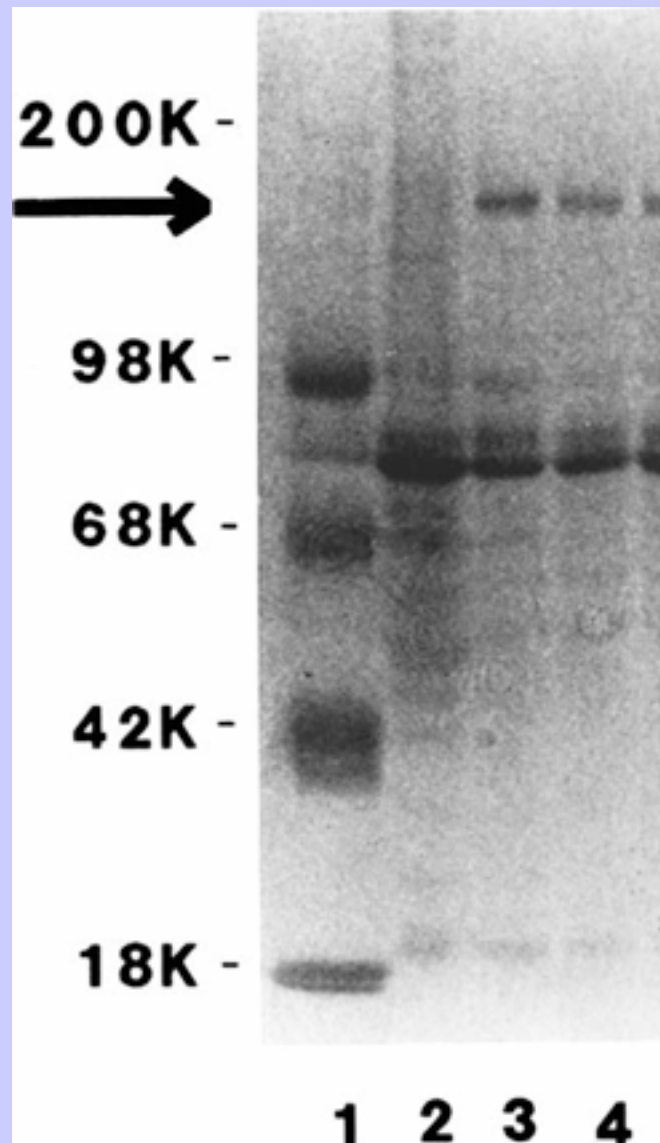


FIGURE 34-4 A Western blot of bovine Mac-1. An extract has been made from the neutrophils of a bovine leukocyte adhesion deficiency (BLAD) calf (*lane 2*) or from clinically normal calves (*lanes 3 and 4*). The extracts have been electrophoresed and blotted onto nitrocellulose. The bands are stained to show the presence of glycoproteins. Note that CD18 (*arrow*) is absent from the lysate of neutrophils from a BLAD calf. Lane 1 shows molecular weight standards (kD). (From Kehrli ME Jr, Schmalstieg FC, Anderson DC, et al: *Am J Vet Res* 51:1826-1836, 1990.)



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perhaps, that it was not effectively removed. In spite of this, these cells were unable to kill opsonized *Escherichia coli* or *Staphylococcus aureus*, suggesting that they had a killing defect rather than an adherence defect.

34.2.4 Bovine Leukocyte Adhesion Deficiency

An integrin deficiency called bovine leukocyte adhesion deficiency (BLAD) also occurs in Holstein calves. It is an autosomal recessive trait characterized clinically by recurrent bacterial infections, anorexia, oral ulceration, gingivitis, periodontitis, chronic pneumonia, stunted growth, delayed wound healing, peripheral lymphadenopathy, and a persistent extreme neutrophilia. Affected calves usually die between 2 and 7 months of age. The survivors grow slowly and may develop amyloidosis. These calves have large numbers of intravascular neutrophils but very few extravascular neutrophils, even in the presence of invading bacteria.

The disease results from a point mutation in the gene coding for CD18 ([Figure 34-3](#)). As a result, an aspartic acid residue is replaced by a glycine, and functional CD18 is not produced. In the absence of this chain, complete integrins cannot form. As a result, neutrophils fail to attach firmly to vascular endothelial cells and cannot emigrate from blood vessels. Healthy carriers have a single copy of the mutated gene and so have abnormally low levels of CD18 ([Figure 34-4](#)). Through the use of a test based on the DNA PCR, the presence of the altered gene can be demonstrated. In this way it has been shown that one bull, Osborndale Ivanhoe, with thousands of registered sons and daughters, was a carrier of this gene. As a result, the defective gene was widespread and common among Holstein cattle in the United States (14% of bulls, 5.8% of cows). Fortunately, carrier animals can now be rapidly de-tected and removed from breeding programs.

Because CD18 integrins are also employed by T cells moving to sites of antigen invasion, BLAD calves show delayed or poor type IV hypersensitivity responses to intradermal skin testing. Their neutrophils show reduced responsiveness to chemotactic stimuli and diminished superoxide production and myeloperoxidase activity. They have increased expression of Fc receptors but decreased binding and expression of C3b and immunoglobulin M (IgM) on neutrophil surfaces, implying an alteration in receptor function. This is reflected by greatly reduced endocytosis and killing of *S. aureus*.

34.2.5 Canine Cyclical Neutropenia

Canine cyclical neutropenia (gray Collie syndrome) is an autosomal recessive disease of border collies. Affected dogs have dilution of skin pigmentation, eye lesions, and regular cyclic fluctuations in leukocyte numbers. Their hair is characteristically a silver-gray color. The loss of neutrophils occurs about every 11 to 12 days and lasts for about 3 days. It is followed by normal or elevated neutrophil counts for about 7 days. Severe neutropenia suppresses inflammation and increases susceptibility to bacterial and fungal infections. (Their neutrophils also have reduced myeloperoxidase activity, so the disease is not entirely due to a neutrophil deficiency.) The nature of the defect is not clear but is probably a result of fluctuations in myeloid stem cell numbers due to abnormalities in growth factor production. Some ultrastructural changes have been observed in neutrophil precursors. The animals have severe enteric disease, respiratory infections, mouth infections (gingivitis), bone disease (arthralgia), and lymphadenitis and rarely live beyond 3 years. Because platelet numbers also cycle, affected dogs may also have bleeding problems, including gingival hemorrhage and epistaxis. Immunoglobulin levels rise as a result of the recurrent antigenic stimulation, but complement levels cycle in conjunction with the neutropenia. The disease begins to express itself as maternal immunity wanes. Affected puppies are weak, grow poorly, have wounds that fail to heal, and have a high mortality. If they are kept alive by aggressive antibiotic therapy, the chronic inflammation may lead to amyloidosis.

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Treatment involves the repeated use of antibiotics to control the recurrent infections. If endotoxin is administered repeatedly, it can stimulate the bone marrow and stabilize neutrophil, reticulocyte, and platelet numbers. Lithium carbonate has a similar effect. Unfortunately, both endotoxin and lithium carbonate are toxic, and the disease recurs when the treatment is discontinued.

34.2.6 Other Examples of Defective Neutrophil Function

An inherited defect in neutrophil bactericidal activity has been reported in Dobermans. Dogs had bronchopneumonia and chronic rhinitis that developed soon after birth and persisted despite antimicrobial therapy. Although their chemotaxis and ingestion were apparently normal, their neutrophils were unable to kill *S. aureus*. Since these cells showed reduced reduction of nitroblue tetrazolium and superoxide production, it was suggested that there was a defect in the respiratory burst pathway.

Young Weimaraner dogs have been described as suffering from an immunodeficiency syndrome with a wide range of clinical signs. These include recurrent fevers, diarrhea, pneumonia, pyoderma, osteomyelitis, stomatitis, and osteomyelitis. They may have defective neutrophil function, as shown by a depressed chemiluminescent response to phorbol ester, implying a defect in the respiratory burst mechanism. Their IgG levels may be significantly lower than normal and their IgM and IgA levels were somewhat low; all the other immunological parameters of these animals were within normal ranges. In many cases the inflammatory responses may develop within a week of receiving a vaccine (see [Chapter 21](#)).

A persistent neutropenia attributable to a deficiency of granulocyte colony-stimulating factor (G-CSF) has been reported in a 3-year-old male Rottweiler. The animal had a fever due to multiple recurrent infections, especially a chronic bacterial arthritis in the presence of a persistent neutropenia. A bioassay showed that the animal was not making any G-CSF. Its myeloid stem cells responded readily to additional G-CSF, suggesting that they were functionally normal. Bone marrow examination suggested that its neutrophil precursors had failed to mature.

A possible autosomal recessive neutropenia has been described in border collies. This disease, called “trapped neutrophil syndrome,” resulted in recurrent bacterial osteomyelitis and gastroenteritis. Animals presented with persistent fever and lameness due to lytic bone lesions. They had myeloid hyperplasia and dense accumulations of neutrophils in the marrow but few in the blood. The neutropenia apparently resulted from an inability of the neutrophils to escape from the bone marrow into the bloodstream, perhaps as a result of a deficiency of GM-CSF. In humans this disease has been called myelokathexis.

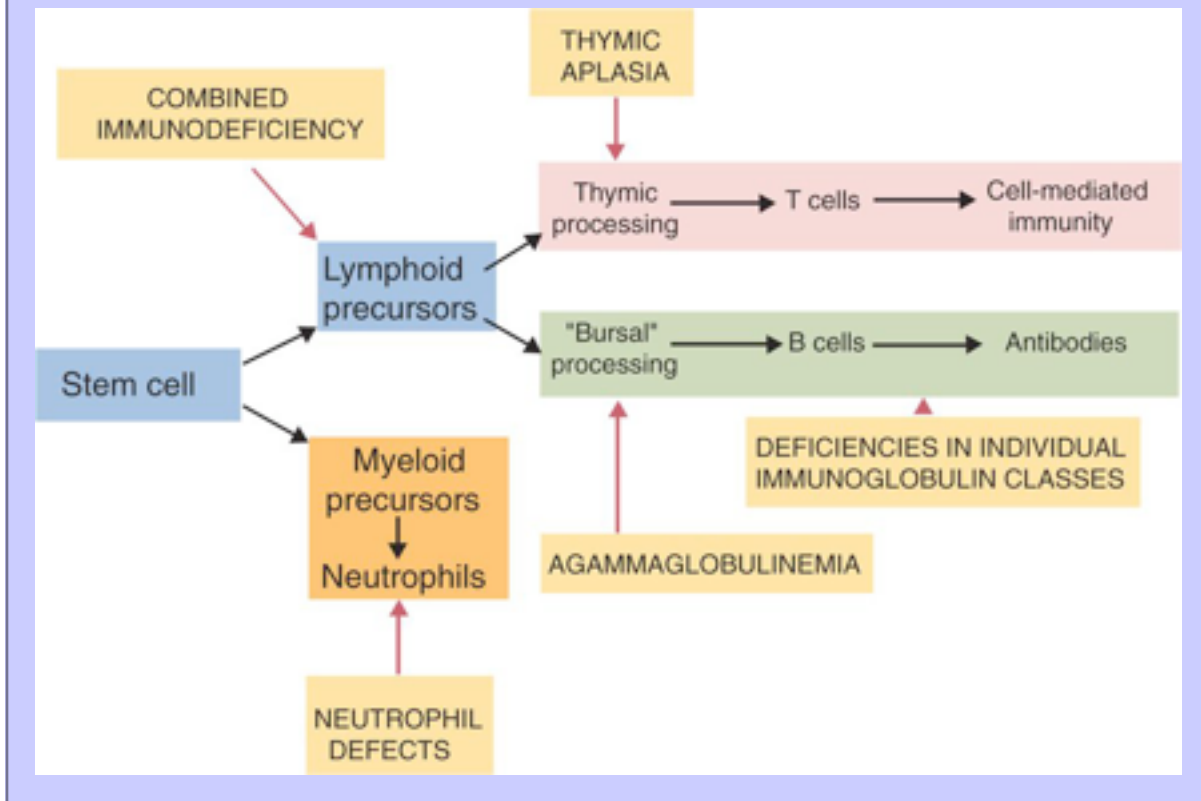
34.3 INHERITED DEFECTS IN THE ACQUIRED IMMUNE SYSTEM

The inherited immunological defects have served to confirm the overall arrangement of the immune system, as outlined in [Figure 34-5](#). For example, if both the cell- and antibody-mediated immune responses are defective, it may be assumed that the genetic lesion operates at a point before thymic and bursal cell processing—that is, the defective response is the result of a stem-cell lesion. A defect that occurs only in thymic development is reflected in an inability to mount cell-mediated immune responses, although antibody production may be normal. Similarly, a lesion restricted to B cells is reflected by impaired antibody responses.

Recent advances in molecular genetics have enabled many new primary immunodeficiency disorders to be identified in humans. For example, at least ten different mutations can result in severe combined immunodeficiency. Each mutation results in a defect that corresponds to a key step in the differentiation of T cells. Likewise, mutations in many different genes can disable B cell function and result in immunoglobulin deficiencies.

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FIGURE 34-5 The points in the immune system where development blocks may lead to immunodeficiencies.



34.4 IMMUNODEFICIENCIES OF HORSES

Horses are among the few domestic animals whose economic worth has permitted a thorough analysis of neonatal mortality. As a result, a significant number of primary immunodeficiency syndromes have been identified in this species ([Figure 34-6](#)).

34.4.1 Severe Combined Immunodeficiency

The most important congenital equine immunodeficiency is the severe combined immunodeficiency syndrome (SCID). Affected foals fail to produce functional T or B cells and have very few circulating lymphocytes. If they suckle successfully, they will acquire maternal immunoglobulins. Once these have been catabolized, however, these foals cannot produce their own antibodies and eventually become agammaglobulinemic. Affected foals are therefore born healthy but begin to sicken by 2 months of age. The precise time of onset depends on the quantity of colostral antibodies absorbed. All die by 4 to 6 months as a result of overwhelming infection by a variety of low-grade pathogens. Severe bronchopneumonia is the predominant presenting sign. Organisms that have been implicated in this bronchopneumonia include equine adenovirus, *Rhodococcus equi*, and *Pneumocystis carinii* (an opportunistic fungal pathogen). The disease is manifested by a nasal discharge, coughing, dyspnea, weight loss, and fevers. Affected foals may also develop enteritis, omphalophlebitis, and many other infections.

Cryptosporidium parvum and many different bacteria have been implicated in the enteritis.

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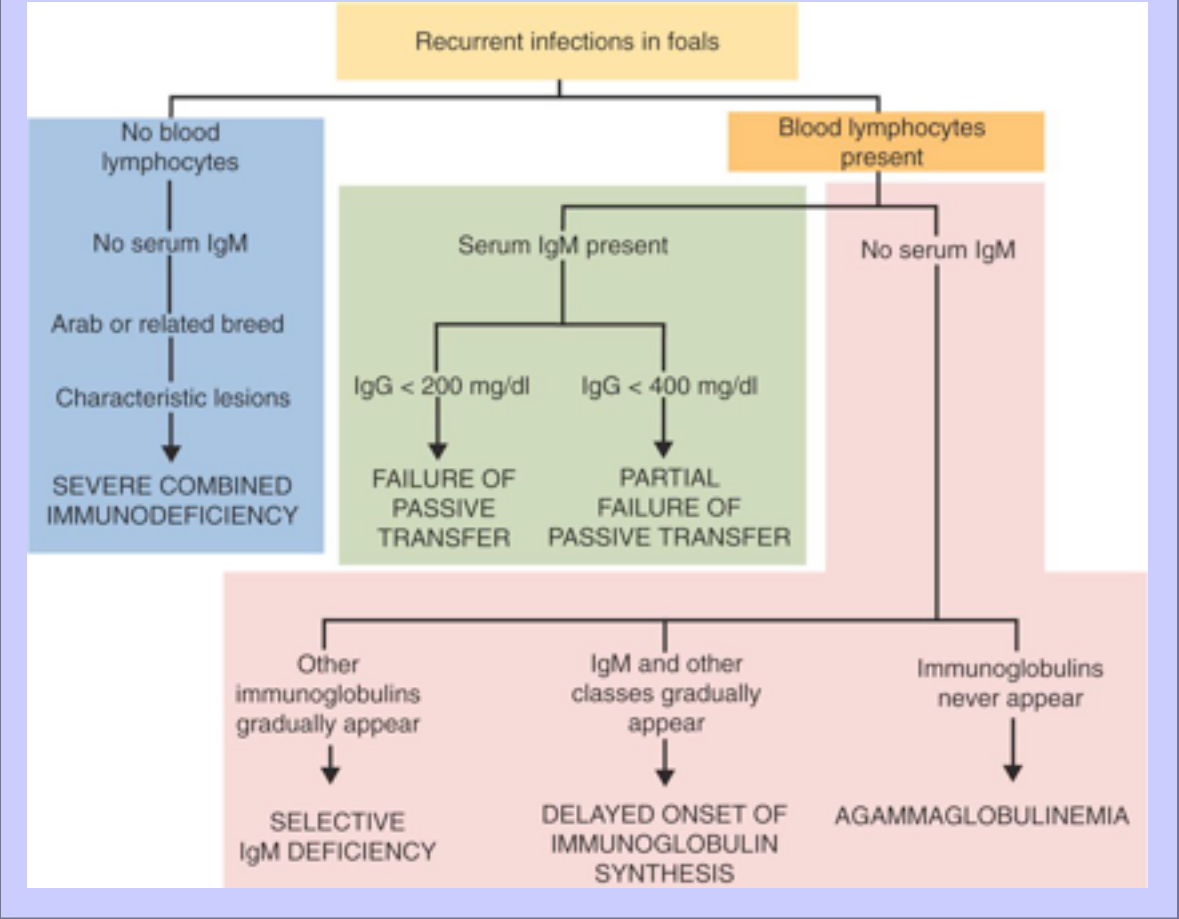
On necropsy, the spleens of these foals lack germinal centers and periarteriolar lymphoid sheaths. Their lymph nodes lack lymphoid follicles and germinal centers, and there is cellular depletion in the paracortex. The thymus in these animals may be difficult to find. By using large quantities of blood, it is possible to demonstrate the presence of functional NK cells. Neutrophil and monocyte functions are also normal in these foals.

SCID is an autosomal recessive disease, and its occurrence therefore indicates that both parents carry the mutation. Accurate diagnosis is of great importance, since the presence of the mutation reduces significantly the value of the parent animals. Thus all suspected cases must be confirmed by postmortem examination. The clinical diagnosis of SCID requires that at least two of the following three criteria be established: (1) very low (consistently below 1000/mm³) circulating lymphocytes; (2) histology typical of SCID—that is, gross hypoplasia of the primary and secondary lymphoid organs; and (3) an absence of IgM from presuckle serum. (The normal equine fetus synthesizes small amounts of IgM. As a result, IgM in normal newborn foals is about 160 mg/ml. If the foal successfully suckles, it will obtain immunoglobulins of all isotypes from the mare's colostrum. However, the half-life of IgM is only about 6 days, so maternal IgM will disappear within a few days of birth. Thus a normal foal will always have some IgM in its serum, but a SCID foal will have none.)

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FIGURE 34-6 The differential diagnosis of the equine immune deficiencies.



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The presence of the mutant CID gene in horses can be detected by means of a PCR procedure. A sample of DNA is obtained from horse skin cells. A set of primers designed to amplify only DNA containing the 5-base pair deletion and another set designed to amplify only the normal allele are used to determine whether the mutant gene is present. This test has demonstrated that the frequency of the SCID gene in Arabian horses is 8.4%. Based on this, it would be expected that 0.18% of Arabian foals would be homozygous for the trait and hence clinically affected. Pedigree analysis suggests that the SCID trait was introduced to the United States by a single stallion in the 1920s.

34.4.1.1

Molecular Basis of Equine SCID

When the antigen receptors (B cell antigen receptors [BCRs] and T cell antigen receptors [TCRs]) are synthesized, large segments of DNA are excised so that V, D, and J gene segments can be rejoined (see [Chapter 15](#)). Several enzymes are involved in this recombination process. Some cut the DNA strands, and others rejoin them. Studies on the cells of SCID foals show that although the enzymes that cut the DNA are normal, there is a defect in the large multicomponent enzyme that rejoins the cut ends. The specific defect lies in the gene coding for the catalytic subunit of an enzyme called DNA-dependent protein kinase (DNA-PKcs) ([Figure 34-7](#)). In the mutant DNA-PKcs gene, a loss of five nucleotides results in a frameshift, premature termination of the peptide chain, and a deletion of 967 amino acids from the C-terminus of the molecule, including its entire kinase domain ([Figure 34-8](#)). Functional DNA-PKcs is totally absent from affected foals. Because of this deficiency, broken DNA strands cannot be rejoined, and neither T cells nor B cells can form functional V regions. In the absence of both TCRs and BCRs, affected foals cannot respond to antigens.

Since DNA-PKcs is needed to rejoin broken strands of DNA, it also plays a key role in other DNA repair processes. Thus when normal foal cells are irradiated, they can repair the damage to DNA caused by the ionizing radiation. The cells from SCID foals, in contrast, are unable to repair their DNA. These cells are much more susceptible to radiation-induced damage ([Figure 34-9](#)).

34.4.2

Immunoglobulin Deficiencies

Primary agammaglobulinemia is a rare disease of foals. Affected animals have no identifiable B cells (cells with surface immunoglobulins) and have very low immunoglobulin levels. Their lymphoid tissues contain no primary follicles, germinal centers, or plasma cells. Nevertheless, their blood lymphocytes can respond

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FIGURE 34-7 The defect in the p350 component of the DNA-dependent protein kinase that prevents DNA repair in severe combined immunodeficiency foals.

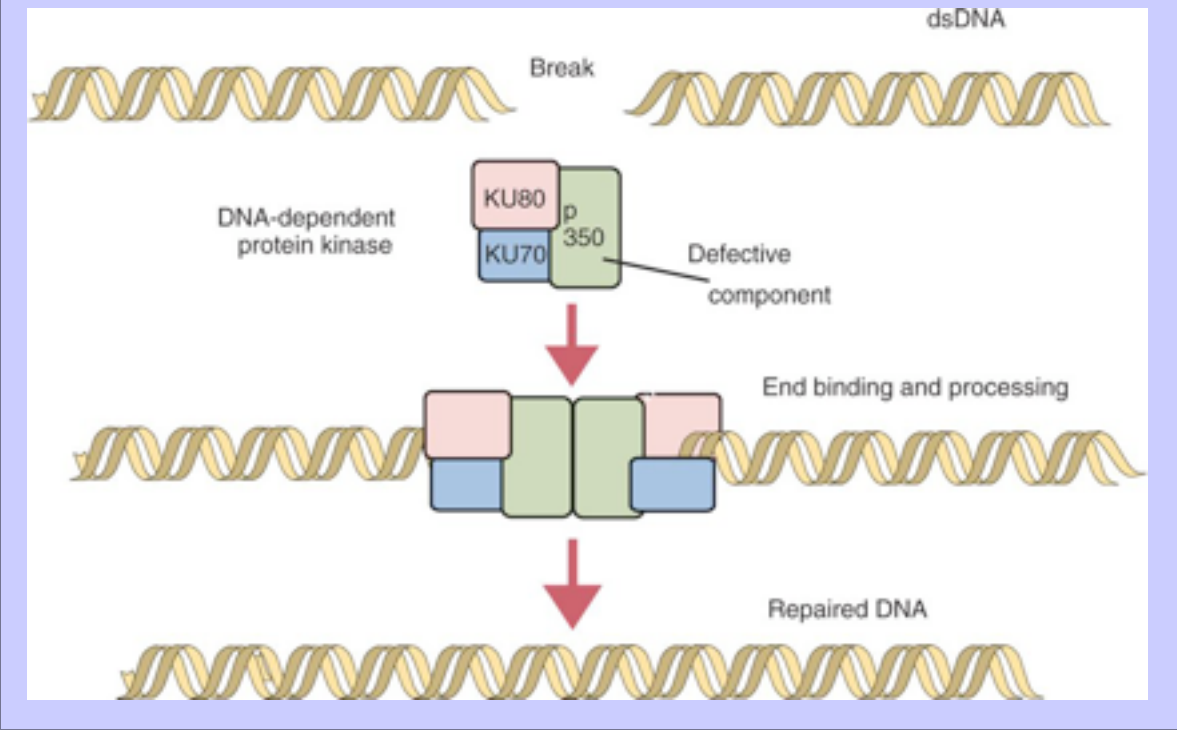
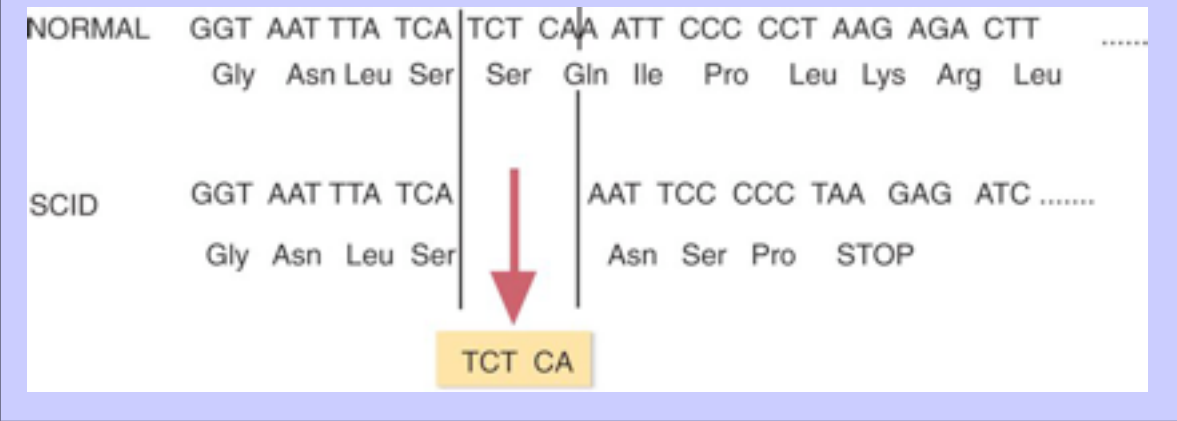


FIGURE 34-8 The gene deletion in the equine DNA-PK p350 gene that leads to premature termination of the molecule.



to mitogens and produce the cytokine migration inhibitory factor. Intradermal inoculation of phyto-hemagglutinin induces a typical type IV delayed hypersensitivity reaction. Affected foals experience recurrent bacterial infections but can survive for 17 to 18 months. The disease should be suspected in a foal having a

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normal lymphocyte count but lacking both IgM and IgG. It may be confirmed by showing normal T cell responses to mitogens and an absence of B cells.

Selective IgM deficiencies have been described in foals. Serum IgM levels in these animals are at least two standard deviations below normal, but IgG and IgA levels and B cell numbers are normal. In most cases, foals have septicemia or recurrent respiratory tract infections, often involving *Klebsiella pneumoniae* or *R. equi*, and die by 10 months of age. Some affected foals live longer and respond to therapy but fail to grow, have recurrent respiratory infections, and die by 24 months of age. Most affected foals have been Arabians or quarter horses, suggesting that the disease may have a genetic basis.

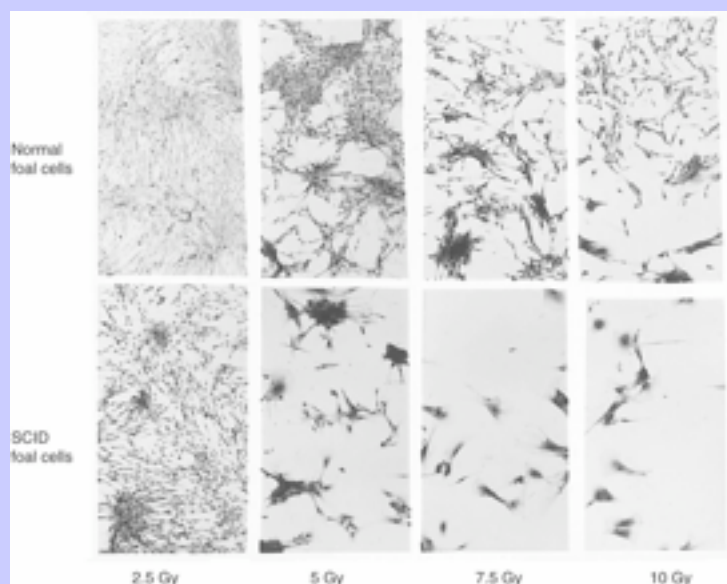
A single case of IgG deficiency has been described in a 3-month-old foal with salmonellosis. The animal had normal IgA and IgM but no germinal centers, lymphoid follicles, splenic follicles, or periarteriolar lymphoid sheaths. Serum IgG was extremely low.

Between 2 and 3 months of age, some foals experience a transient hypogammaglobulinemia as a result of a delayed onset of immunoglobulin synthesis. These animals may have recurrent infections during the period when their immunoglobulin levels are low. Lymphocyte numbers and responsiveness remain normal at this time.

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FIGURE 34-9 The effect of radiation on normal foal fibroblasts and on fibroblasts from a severe combined immunodeficiency (SCID) foal. Equivalent numbers of cells were exposed to varying amounts of ionizing radiation as indicated and cultured in chamber slides. Five days later the slides were fixed, stained, and photographed. Note that there are many fewer SCID cells surviving this treatment since they are unable to repair their DNA. (From Wiles R, Leber R, Moore BB, et al: *Proc Natl Acad Sci U S A* 93:11485-11489, 1995. Courtesy Dr. K. Meek.)



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34.4.3 Common Variable Immunodeficiency

Common variable immunodeficiency (CVID) is the second most common primary immunodeficiency disease in humans (after selective IgA deficiency). It is a heterogeneous group of sporadic diseases all characterized by a failure of B cells to make antibodies. In most cases, the B cell deficiency is secondary to a defect in helper T cells. Unlike the other primary immunodeficiencies, most cases are diagnosed in adults.

Some human cases have been attributed to a deficiency of the co-stimulator molecule ICOS. This molecule is expressed on activated T cells and is required for cytokine release and co-stimulation of B cells. ICOS deficiency therefore prevents appropriate stimulation of B cell responses. Only about 5% of human patients with CVID have an ICOS deficiency and no studies on ICOS in animals have been reported.

Cases of CVID have been recorded in horses. Although they resemble primary immunodeficiencies in their sporadic nature and severity, they usually occur in animals older than 3 years of age. Typically, the horses present with recurrent infections that are not responsive to medical treatment. Bacterial meningitis may be a consistent feature. Their serum contains only trace levels of IgG and IgM, no detectable IgG3, and very low IgA levels. Sometimes individual IgG subclasses are deficient while IgA levels are normal. T cell numbers are normal, but B cells are undetectable and there is no response to the B cell mitogen lipopolysaccharide. On necropsy, there are no B cells in lymphoid organs, blood, or bone marrow. Some horses may have severe liver disease, a feature also seen in humans. It is suspected that these individuals have an underlying defect that is only expressed when the immune system is stressed by infection. Other cases have included horses between 2 and 5 years old with a selective IgM deficiency. Many develop a concurrent lymphosarcoma, and limited evidence suggests that they have excessive suppressor T cell function.

34.4.4 Fell Pony Immunodeficiency Syndrome

An autosomal recessive syndrome consisting of anemia, peripheral ganglionopathy, and immunodeficiency has been reported in Fell pony foals. Affected foals appear normal at birth but fail to thrive. The disease develops at 4 to 12 weeks of age as maternal immunity wanes. The foals become extremely anemic and suffer from opportunistic infections such as diarrhea or respiratory disease. The foals usually die or are euthanized by 3 months of age as a result of infections with *Cryptosporidium* or adenoviruses. Affected animals lack germinal centers and lack plasma cells, suggesting some form of B cell deficiency. Indeed, B cell numbers in affected foals are less than 10% of normal levels, although CD4 and CD8 T cell numbers are within the normal range. Immunoglobulin levels are relatively normal, but any immunoglobulin deficiency may be masked by maternal antibodies. Neutrophil counts are normal—their lymphocytes show reduced major histocompatibility complex (MHC) class II expression. The molecular basis of the syndrome is unknown.

34.4.5 Incidence of Immunodeficiencies

The most important immunodeficiency in foals is not inherited but results from a failure to absorb sufficient colostral antibodies from the mare (see [Chapter 18](#)). This failure of passive transfer may affect up to 10% of all foals. SCID occurs in 2% to 3% of Arab foals and is 10 times more common than selective IgM deficiency. Selective IgM deficiency is, in turn, 10 times more common than agammaglobulinemia.

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34.5 IMMUNODEFICIENCIES OF CATTLE

34.5.1 Severe Combined Immunodeficiency

A combined immunodeficiency has been recorded in an Angus calf. The animal was apparently normal when born and suckled normally. It became ill, however, at 6 weeks of age when it developed pneumonia and diarrhea. The animal was lymphopenic and severely hypogammaglobulinemic. It had undetectable IgM and IgA and a low level of IgG, which was believed to be the residue of passively acquired maternal antibodies. The animal died within a week with systemic candidiasis. It had a hypoplastic thymus consisting of epithelial cells but no thymocytes. It had no detectable lymph nodes and a hypoplastic spleen that had no lymphocytes within its periarteriolar lymphoid sheaths. The syndrome thus closely resembled equine SCID.

34.5.2 Selective IgG2 Deficiency

IgG2 deficiency has been reported in red Danish cattle. About 1% to 2% of this breed is completely deficient in this immunoglobulin subclass and as a result has increased susceptibility to pneumonias and gangrenous mastitis. Up to 15% may also have low IgG2 levels, although they do not appear to have any ill effects in consequence.

34.5.3 Hereditary Parakeratosis

Certain Black Pied Danish and Friesian cattle carry an autosomal recessive trait of thymic and lymphocytic hypoplasia (Trait A-46). Affected calves are born healthy, but by 4 to 8 weeks they begin to experience severe skin infections. If untreated, they die within a few weeks, and none survives for longer than 4 months. Affected calves have exanthema, hair loss on the legs, and parakeratosis around the mouth and eyes. There is depletion of lymphocytes in the gut-associated lymphoid tissue and atrophy of the thymus, spleen, and lymph nodes. These animals are T cell deficient and have depressed cell-mediated immunity but normal antibody responses. Thus they mount a normal antibody response to tetanus toxoid but respond poorly to dinitrochlorobenzene or tuberculin, both of which induce cell-mediated reactions. If these calves are treated by oral zinc oxide or zinc sulfate, they recover the ability to mount normal cell-mediated responses. However, if the zinc supplementation is stopped, the animals will relapse within a few weeks. It is probable that these animals have a reduced ability to absorb zinc from the intestine. Zinc is an essential component of the thymic hormone thymulin (see [Chapter 36](#)) and is therefore required for a normal T cell response.

34.5.4 Other Immunodeficiencies

A transient hypogammaglobulinemia associated with a delayed onset of immunoglobulin synthesis has been recorded in a Simmental heifer, and a case of thymic aplasia with absence of hair has been described in calves. It is probably similar to the “nude” mutation seen in mice and cats (p. 461).

34.6 IMMUNODEFICIENCIES OF DOGS

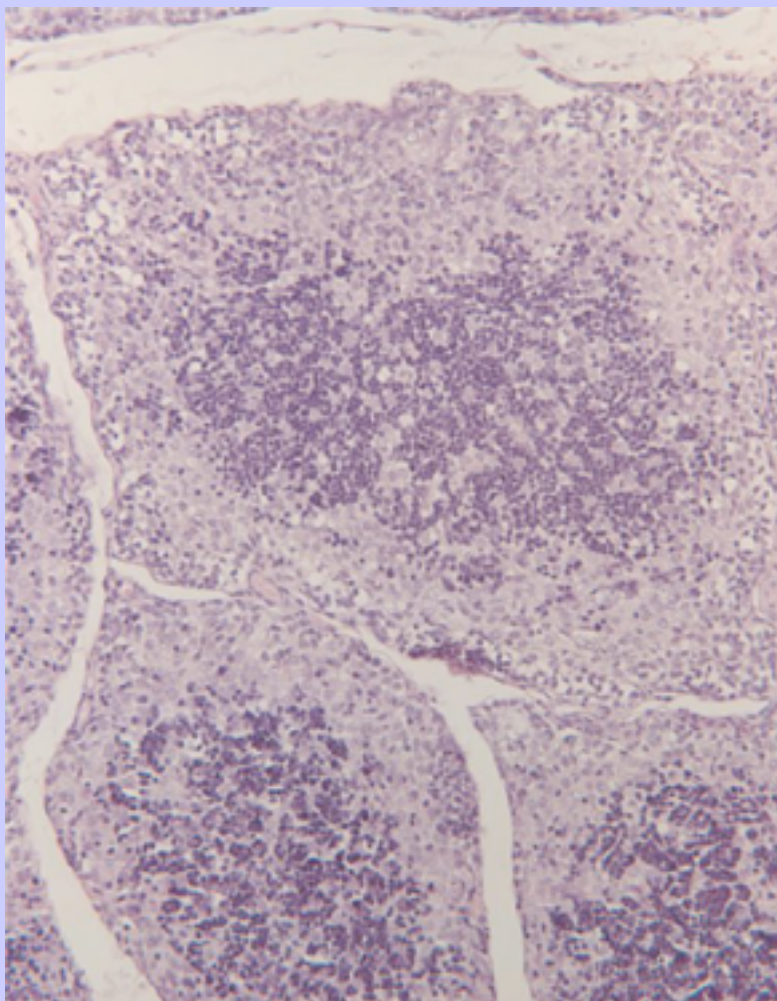
34.6.1 Combined Immunodeficiencies

A SCID resulting from a defect in the catalytic subunit of the DNA-dependent protein kinase (DNA-PKcs) has been identified in Jack Russell Terriers. From a single breeding pair of terriers, 12 of 32 siblings died from opportunistic infections between 8 and 14 weeks of age. These animals showed a SCID phenotype with lymphopenia, agammaglobulinemia, and thymic and lymphoid aplasia. The disease appeared to be inherited

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FIGURE 34-10 Photomicrograph of the thymus of a basset hound with X-linked immunodeficiency. Note the lack of a defined cortex and the scattered foci of dark-staining lymphocytes. (H&E stain.) (From Snyder PW, Kazacos EA, Felsburg PJ: *Clin Immunol Immunopathol* 67:55-67, 1993.)



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as an autosomal recessive condition. It resulted from a point mutation, leading to stop codon formation and premature termination of the peptide chain 517 amino acids before the normal C-terminus. Affected dogs showed severely diminished expression of DNA-PKcs. As in equine SCID, the defect blocks gene splicing during V(D)J recombination in TCR and immunoglobulin variable regions. The carrier frequency of this gene is 1.1%.

An X-linked SCID has been recorded in basset hounds and Cardigan Welsh Corgis. The disease is characterized by stunted growth, increased susceptibility to infections, and absence of lymph nodes. Clinically, animals are healthy during the immediate neonatal period as a result of maternal antibodies. However, by 6 to 8 weeks, as the levels of maternal antibodies decline, the animals begin to develop infections. At first these are relatively mild infections, such as superficial pyoderma and otitis media. Eventually, they become more severe, and untreated animals die of severe pneumonia, enteritis, or sepsis by 4 months of age. Common infections include canine distemper, generalized staphylococcal infections, adenoviral and parvoviral infections, and cryptosporidiosis. It is interesting to note that *P. carinii* pneumonia has not been recorded in these dogs. This immunodeficiency is an X-linked disorder since breeding of a carrier female to a normal sire results in approximately half the males in each litter being affected and all the females being phenotypically normal.

On examination these dogs usually have reduced blood lymphocyte numbers ($\approx 1000/\mu\text{l}$) but are not profoundly lymphopenic. Their CD4/CD8 ratio is, however, approximately 15 : 1 as compared with normal dogs, which have a CD4/CD8 ratio of 1.7 : 1. This indicates a major drop in CD8⁺ cell numbers. The absolute number of T cells persists at less than 20% of normal. The dogs have normal numbers of B cells. The few lymphocytes in the blood are unresponsive to mitogens. The puppies have normal IgM levels but very low, or no, IgG and IgA. The dogs do not make antibodies against antigens such as tetanus toxoid.

On necropsy the thymus of affected dogs is approximately 10% of the normal weight and lacks a defined cortex ([Figure 34-10](#)). The total number of thymocytes is approximately 0.3% of normal. The lymph nodes and tonsils of affected dogs are very small and dysplastic and may be very difficult to find. When present, the nodes are disorganized and contain very few small lymphocytes. Their spleens contain large periarteriolar lymphoid nodules with occasional small lymphocytes and few plasma cells. The bone marrow in these dogs appears normal. Approximately 40% of the thymocytes of these dogs are CD4⁺CD8⁻, compared with 16% of the thymocytes of normal dogs.

The disease results from a mutation in the gene coding for the γ chain of interleukin-2R (IL-2R) (IL-2R γ). The same chain is also a component of the IL-4, IL-7, IL-9, and IL-15 receptors and has been designated the common γ -chain, γc . In affected Basset Hounds, a loss of four bases in the γc gene causes a frameshift. As a result of this frameshift, a stop codon is generated. Thus, instead of the complete protein, only a small peptide is produced and no functional protein is made. A second SCID mutation has been described in Cardigan Welsh Corgis. In these animals a single cytosine residue is inserted into the γc gene so that a stop codon is generated before the transmembrane domain, resulting in a failure to synthesize the complete chain ([Figure 34-11](#)). As a result this peptide is not expressed on the cell surface. In both cases, the mutation does not interfere with IL-2 production, but the lymphocytes of these animals are unresponsive to IL-2. In the absence of a γc chain, mature T cells will not develop.

Experimentally, affected dogs may be “cured” by bone marrow allografts. Reconstitution with normal bone marrow results in the presence of normal donor T cells and a mixed chimerism ranging from 30% to 50% donor B cells.

34.6.2 Immunoglobulin Deficiencies

A selective IgM deficiency has been reported in two related Doberman Pinschers. One animal was asymptomatic,

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FIGURE 34-11 The two defined canine X-linked severe combined immunodeficiency (SCID) mutations in the interleukin-2R γ (IL-2R γ) gene. In the corgi mutation, the insertion of a single cytosine residue into the gene leads to the generation of a stop codon and premature termination of peptide synthesis. In the basset mutation, deletion of four bases causes a frameshift mutation and also leads to the generation of a stop codon (not shown). (Data from Henthorn PS, Somberg RL, Fimiani VM, et al: *Genomics* 23:69-74, 1994; and from Somberg RL, Pullen RP, Casal ML, et al: *Vet Immunol Immunopathol* 47:203-214, 1995.)



whereas the other had a chronic mucopurulent nasal discharge and bronchopneumonia. Both these animals had raised IgA, low IgG, and very low IgM. They experienced only a chronic nasal discharge, so the clinical significance of this deficiency is in doubt.

Selective deficiencies of IgA have been observed in several breeds of dogs, but German Shepherds are especially predisposed to a range of infectious disorders, including mycoses, anal furunculosis, deep pyoderma, and small intestinal bacterial overgrowth. This suggests that they may have deficiencies in mucosal immunity. Consistent with this is the observation that German Shepherds in the United Kingdom have normal IgM and IgG levels but significantly reduced levels of IgA (≈ 80 mg/dl, as opposed to 170 mg/dl in the control group). Likewise, dogs of

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this breed have significantly lower concentrations of IgA in their tears as compared with other breeds. They have normal numbers of IgA-producing plasma cells, implying that the deficiency may be due to defective synthesis or secretion of IgA. German Shepherd dogs have significantly reduced median IgA levels in fecal extracts as compared to control dogs of other breeds. Many have IgA concentrations below the 95% confidence limit of the control population, while many lack detectable fecal IgA. Their fecal IgG and albumin levels tend to be higher than controls, suggesting a mild enteritis.

Shar-Pei puppies with recurrent cough, nasal discharge conjunctivitis, and pneumonia, as well as demodicosis and *Microsporum canis* infections, have been identified as having a selective IgA deficiency (<15 mg/dl). Likewise, abnormally low IgA concentrations have been found in a high percentage of clinically normal Shar-Pei dogs. A high incidence of atopic disease is observed in these dogs, a feature also seen in IgA-deficient humans.

A primary selective IgA deficiency has been described in an inbred beagle colony. The colony had a history of parainfluenza and endemic kennel cough due to *Bordetella bronchiseptica*. Despite vaccination, these animals continued to experience recurrent respiratory tract infections and otitis. Immunoelectrophoresis and radial immunodiffusion showed that affected dogs had normal serum IgG and IgM levels but very little IgA (<5 mg/dl). Phenotypically normal parent dogs had very low IgA levels. Four affected dogs had circulating anti-IgA antibodies. Their T and B lymphocyte numbers and lymphocyte responses to mitogens were normal, as was their response to tetanus toxoid. They had a normal number of plasma cells secreting IgG and IgM but no plasma cells secreting IgA. When two affected animals were mated, four out of five pups in a litter were IgA deficient. The disease was not sex-linked.

A transient hypogammaglobulinemia has been seen in two animals from a litter of Spitz puppies that experienced recurrent upper respiratory tract infections between 8 and 16 weeks of age. These dogs had normal T cell numbers and mitogen responses. They had low immunoglobulin levels and low antibody titers to vaccine antigens at 16 weeks. These puppies responded very weakly to tetanus toxoid when it was administered at 4 months. By 6 months, however, immunoglobulins had risen to normal levels and the puppies regained their health. It is believed that these puppies experienced a delayed onset of immunoglobulin synthesis. Symptomatic treatment is sufficient to carry these animals until their immune system becomes functional.

Cavalier King Charles Spaniels with *Pneumocystis* pneumonia have IgG concentrations that were significantly lower in infected dogs (median 3.2 mg/ml) than in breed and age-matched control dogs (median 8.5 mg/ml). IgM levels, in contrast, were significantly higher in the affected dogs. IgA levels were within the normal range. Lymphocyte counts in affected dogs were normal or high. It appears that this may well be an IgG deficiency syndrome.

P. carinii pneumonia has been observed repeatedly in young miniature Dachshunds. The affected animals are usually less than 1 year old and appear to be immunodeficient. Serum electrophoresis shows a marked reduction in IgM, IgG, and IgA. In addition, lymphocyte responses to both phytohemagglutinin and pokeweed mitogens are severely depressed. There is a reduction in B cell numbers. Although the *Pneumocystis* pneumonia responds to aggressive therapy, these animals rarely do well and often die young.

34.6.3

T Cell Deficiencies

A family of inbred Weimaraner dogs has been reported as having immunodeficiency and dwarfism. The animals appeared normal at birth, but at 6 to 7 weeks of age they developed a wasting syndrome characterized by emaciation and lethargy. The dogs began to experience recurrent infections that eventually killed them. On necropsy their thymuses were atrophied and lacked a cortex. These animals had normal immunoglobulin levels,

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their helper cell activity was unimpaired, and their secondary lymphoid organs appeared normal. The lymphocytes of these dogs were unresponsive to mitogens. Growth hormone treatment caused thymic cortical regeneration and a dramatic clinical improvement. However, growth hormone did not restore lymphocyte responsiveness to mitogens. The disease is almost certainly due to a deficiency of growth hormone as a result of a lesion in the hypothalamus and confirms that the thymus requires growth hormone to function.

Bull Terriers suffer from lethal acrodermatitis. This is a complex immunodeficiency syndrome associated with growth retardation, skin lesions (acrodermatitis, chronic pyoderma, paronychia), diarrhea, recurrent pneumonia, and abnormal behavior. The puppies were weak at birth and did not nurse well. Some showed a lighter pigmentation than their littermates. When weaned, they had difficulty eating and failed to grow. Small, crusted lesions developed between the digits and a pustular dermatitis developed around the eyes and mouth at 6 to 10 weeks. The lesions developed into a severe pyoderma. Fungi such as *Malassezia* and *Candida* were readily isolated from the lesions. Diarrhea developed early in the disease, and respiratory tract infections were common. The puppies became depressed and sluggish and died by 15 months of age, with a median survival of 7 months. They had a neutrophilia, normal IgG and IgM levels but significantly lower IgA levels, and hypercholesterolemia. Plasma zinc levels were unusually low. They showed depressed lymphocyte mitogen responses. On necropsy there was a severe loss of T cells so that the puppies lacked a thymus, and the lymph nodes and spleen were very small. The disease is inherited as an autosomal recessive disease, and the parents of affected puppies could be traced to one common ancestor. Because of its similarities to trait A-46 of cattle, these dogs were treated with oral zinc (p. 457). Very high doses resulted in some clinical improvement, but this could not be sustained.

German Shepherd dog pyoderma is, as its name implies, a chronic skin disease that occurs in middle-aged German Shepherd dogs associated with infection by coagulase-positive staphylococci. These cases do not respond well to antibiotic therapy and are believed to reflect some form of underlying genetic or immunological defect. Although affected dogs appear to mount normal humoral responses, limited studies have shown reduced lymphocyte responses to mitogens, an imbalance of lymphocyte subsets (CD4 cells are depressed, CD8 cells are increased), and a decline in the level of CD21⁺ B cells. (The complement receptor CD21 plays a role in B cell activation.) When the number of CD3⁺ T cells and B cells were examined in normal dog skin and in the skin of dogs with deep pyoderma, it was found that the B cell numbers were similar but that the number of T cells infiltrating the lesions in German Shepherd dogs was significantly reduced. Studies on T cell function in these animals have also demonstrated a functional defect. This suggests that T cell dysfunction may play a role in the pathogenesis of deep pyoderma in this breed.

34.6.4

Uncharacterized Immunodeficiencies

The veterinary literature contains several reports of dogs with severe recurrent infections caused by organisms that are not normally considered to be highly pathogenic. Protothecosis has been recorded in dogs. One third of the cases have been in collies, suggesting an inherited predisposition. Weimaraners are unusually susceptible to some systemic bacterial infections; German Shepherds are susceptible to generalized systemic *Aspergillus* infections; and some Rottweiler and Doberman families are unusually susceptible to parvovirus infection. None of these has been shown to be due to primary immunodeficiencies, and all require further investigation.

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34.7 IMMUNODEFICIENCIES OF CATS

34.7.1 Hypotrichosis with Thymic Aplasia

The nude mouse has long been accepted as an important mouse model of immunodeficiency. Nude mice are a strain of hairless mice that fail to develop a functional thymus. This disease has been described in rats, guinea pigs, and calves. A similar mutation has been described in Birman kittens. These kittens were born without any body hair ([Figure 34-12](#)). On necropsy they also had no thymus and had depletion of lymphocytes in the paracortex of lymph nodes, spleen, and Peyer's patches. Thus they were effectively T cell-deficient. Analysis of the pedigree suggested that the disease was inherited as an autosomal recessive disease.

34.8 IMMUNODEFICIENCIES OF MICE

34.8.1 Nude Mice

The best-known mouse model of immunodeficiency is the nude mouse. Nude mice are a strain of hairless mice whose thymic epithelial cells are nonfunctional as a result of a defect in the gene for a transcription factor called FoxN1. (Similar mutations have been observed in rats, guinea pigs, calves, and cats.) Because their thymic epithelial cells fail to function, the primitive thymus in nude mice develops into cysts with walls of immature epithelial cells that do not produce mature T cells. They do possess a limited number of immature T cells and B cells so that a few lymphocytes may be found in peripheral blood. Normal thymus grafts, by restoring epithelial-cell function, permit the T cells of nude mice to mature and develop immune competence. Nude mice are deficient in conventional cell-mediated immune responses, as reflected by prolonged allograft survival and lack of responses to T cell mitogens. Their IgG and IgA levels also are depressed, presumably as a result of a loss of helper T cells.

Although nude mice show enhanced susceptibility to virus-induced tumors, they fail to develop more than the normal level of spontaneous tumors. This observation was, for many years, a major objection to the immunological surveillance theory, because if T cells destroy tumors, T cell-deficient animals should have an increased incidence of neoplasia. However, nude mice possess normal numbers of NK cells, which may protect them in the absence of T cells.

34.8.2 Severe Combined Immunodeficiency Mice

Scid mice have very low numbers of B cells and T cells. Development of B cells is halted before expression of cytoplasmic or cell-membrane immunoglobulins. T cell development is also arrested at a very early stage, and those lymphocytes that do reach the bloodstream are CD4⁻CD8⁻. They have no immunoglobulins and are unable to mount cell-mediated immune responses. *Scid* mice survive relatively well for about a year in specific-pathogen-free facilities but eventually die of *P. carinii* pneumonia. The defects in *scid* mice result from an inability to rearrange their BCR or TCR V region genes correctly. Several different mutations in the DNA joining enzymes have been identified. As a result the cells cannot produce functional receptors, and no functional T or B cells are produced. As in SCID horses, the mouse *scid* mutations also increase sensitivity to ionizing radiation since these animals are unable to repair DNA damage. About 15% of *scid* mice are “leaky” so that they have low levels of immunoglobulins of limited heterogeneity and can reject allografts. Antigen-presenting cells, myeloid and erythroid cells, and NK cells are normal in *scid* mice.

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34.8.3 Moth-Eaten Mice

Moth-eaten mice have a defective T cell system but produce excessive quantities of immunoglobulins and

FIGURE 34-12 Kittens born with an autosomal recessive form of congenital hypotrichosis with thymic aplasia—nude kittens. (From *J Am Anim Hosp Assoc* 30:601, 1994. Courtesy Dr. M.J. Casal.)



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develop autoimmune disease. Their name comes from their appearance. Within a few days of birth, neutrophils invade their hair follicles and cause patchy loss of pigment. These animals lack cytotoxic T cells and NK cells. Mice that are *me/me* have a short life span and usually die as a result of lung damage. The thymus of these animals involutes unusually early, and the emigration of prethymocytes into the thymus is impaired. The B cell hyperactivity may be due to excessive production of some B cell-stimulating cytokines.

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34.8.4 X-Linked Immunodeficiency

Xid mice have a recessive, X-linked B cell defect, making them unable to respond to certain T-independent carbohydrate antigens. They lack certain B cell subsets. Mice that are *bg/nu/xid* are severely immunosuppressed, since they lack T, B, and NK cells. Lightly irradiated *bg/nu/xid* mice can accept human bone marrow xenografts.

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34.9 IMMUNODEFICIENCIES OF HUMANS

Many different immunodeficiency syndromes have been reported in humans. It is anticipated that investigators will succeed eventually in identifying most of these syndromes in domestic animals as well.

The most important phagocytic deficiency syndrome of humans is chronic granulomatous disease. This has not yet been reported as occurring in domestic animals, although it undoubtedly does. Children affected with chronic granulomatous disease have recurrent infections characterized by the development of septic granulomata in lymph nodes, lungs, bones, and skin. The neutrophils of these children are less capable than normal cells of destroying organisms such as staphylococci and coliforms. Their specific lesion is a defect in one of the subcomponents of the NADPH oxidase complex.

Infants suffer from several different forms of combined immunodeficiency. The most severe is reticular dysgenesis, which results from a defect in the development of both myeloid and lymphoid stem cells. Other combined immunodeficiencies result from defects in the development of both T and B lymphoid stem cells. Some of these CID cases are due to a deficiency of the enzyme adenosine deaminase. In other cases there is a defect in the genes coding for IL-2 or IL-7 receptors, for recombinase-activating gene proteins, for CD25, for the CD3g chain, or for MHC class I or class II molecules. The standard treatment for all these diseases is a bone marrow allograft.

34.9.1 T Cell Deficiencies

The DiGeorge anomaly results from a failure of the third and fourth thymic pouches to develop. In consequence, no thymic epithelial tissue develops and few cells populate the T-dependent areas of the secondary lymphoid tissues. Since affected individuals have no functional T cells, they can neither mount a delayed hypersensitivity reaction nor reject allografts. The importance of T cells in protection against viruses is emphasized by the observation that infants with the DiGeorge anomaly generally die of virus infections but remain resistant to bacteria.

34.9.2 B Cell Deficiencies

The most severe of the B cell deficiencies, called Bruton-type agammaglobulinemia, is an X-linked re-cessive disease. Affected infants are devoid of all immunoglobulin classes. They experience recurrent infections due to bacteria such as pneumococci, staphylococci, and streptococci but are usually resistant to viral, fungal, and protozoan infections. The disease results from a mutation in a receptor tyrosine kinase. Inherited deficiencies of individual immunoglobulin classes have also been recorded in humans. As might be anticipated, there are many possible combinations of deficiencies in IgG, IgM, IgA, and IgE, and a tendency to give each a specific name leads to confusion. One of the most important of these is the Wis-cott-Aldrich syndrome. In this disease, a selective IgM deficiency is associated with multiple infections, eczema, and thrombocytopenia. Another such syndrome is ataxia-telangiectasia, in which serum IgA and IgE levels are extremely low or absent and cerebellar and cutaneous abnormalities exist. Affected children, lacking an effective surface immune system, have recurrent bacterial respiratory tract infections. Ataxia-telangiectasia results from a defect in DNA repair mechanisms. In another disease called hyper-IgM syndrome, a defect in the CD40-ligand leads to a failure in the IgM switch so that affected individuals have high levels of IgM but no other antibody classes.

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34.10 IMMUNODEFICIENCIES OF CHICKENS

Birds of the hypothyroid OS strain have a selective IgA deficiency. Birds of the UCD 140 line have a selective IgG deficiency called hereditary dysgammaglobulinemia. These birds have normal immunoglobulin levels for about 50 days after hatching; then their IgG drops and their IgM and IgA rises. In addition to hypogammaglobulinemia, UCD 140 strain birds develop immune-complex lesions, and it has been suggested that a vertically transmitted virus mediates this disease.

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³⁵ CHAPTER 35 Secondary Immunological Defects

^{35.1} KEY POINTS

- Immunodeficiencies induced by some known cause are not uncommon in domestic animals.
- The most important causes of immunosuppression are viral infections. In order to survive within a host, viruses may cause profound immunodeficiency either by infecting and killing lymphocytes or by causing them to become cancerous.
- Other major causes of immunodeficiencies include stress, both physical and mental, some toxins, malnutrition, and old age.

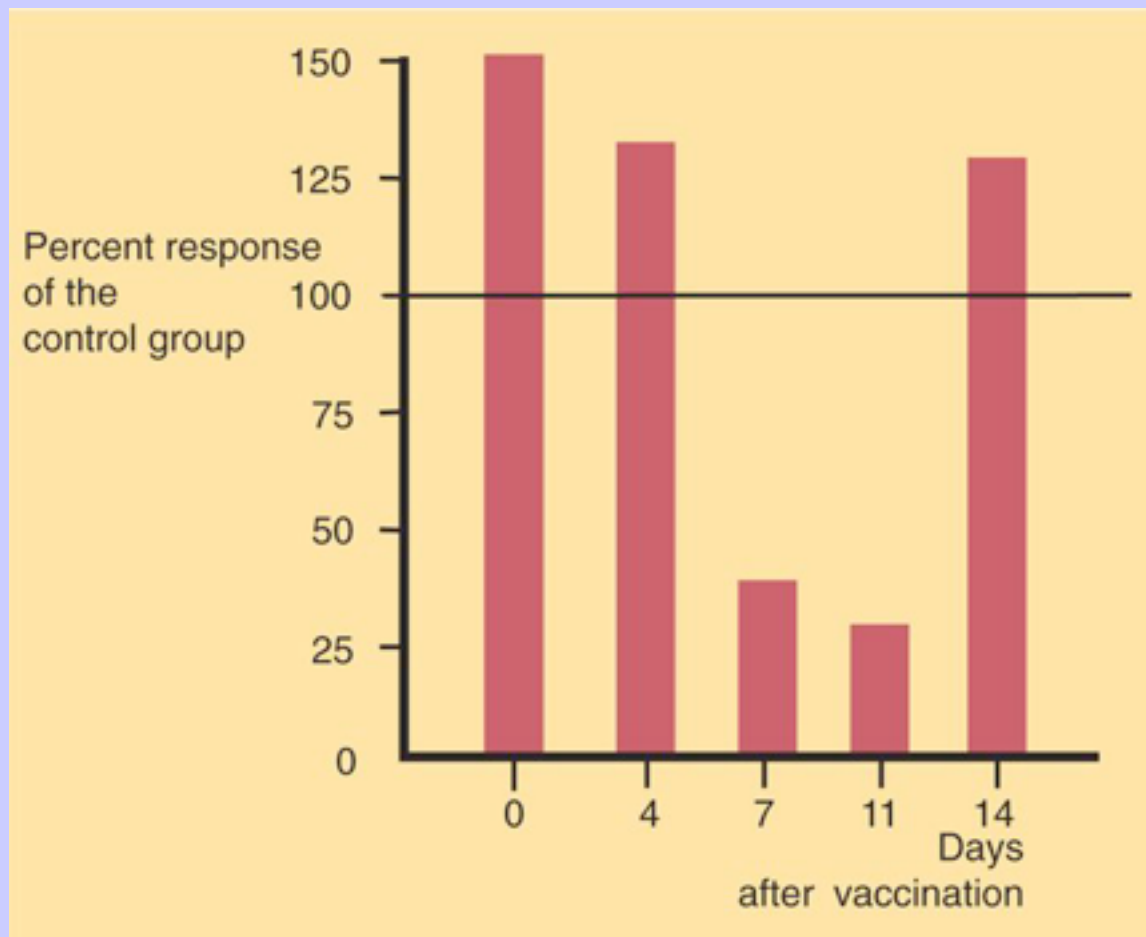
The immune system, like any body system, is subject to destruction and dysfunction as a result of attacks by pathogenic and environmental agents. Among the most important of these agents are microorganisms, especially viruses, toxins, stress of various types, old age, and malnutrition.

^{35.2} VIRUS-INDUCED IMMUNOSUPPRESSION

Viruses that affect the immune system may be divided into those that affect primary lymphoid tissues and those that affect secondary lymphoid tissues. Both types of virus can cause profound immunodeficiencies. For example, in chickens, the infectious bursal disease virus (IBDV) destroys lymphocytes in the bursa of Fabricius. IBDV is not completely specific for bursal cells; it also destroys cells in the spleen and thymus. The spleen and thymus usually recover, whereas the bursa atrophies. The resulting immunosuppression, as might be predicted, is most evident in young birds infected soon after hatching, at a time when the bursa is actively engaged in generating B cells.

One of the most important animal viruses that infects and destroys secondary lymphoid organs

FIGURE 35-1 The immunosuppressive effect of viruses. The effect of administering a mixed vaccine (containing canine distemper, canine adenovirus, canine parainfluenza, canine parvovirus-2, and leptospira) on the response of a puppy's lymphocytes to the mitogen phytohemagglutinin. Control levels were 100%. (From Phillips TR, Jensen JL, Rubino MJ, et al: *Can J Vet Res* 53:154-160, 1989.)



leading to a severe immunodeficiency is canine distemper. Canine distemper virus, although it can multiply in many different cell types, has a predilection for lymphocytes, for epithelia, and for nervous tissue. Its primary cellular receptor is CD150, expressed on activated B and T cells. The distemper virus spreads from its initial invasive sites in the tonsils and bronchial lymph nodes to the bloodstream, where it kills both T and B cells and causes a lymphopenia. Subsequently it invades secondary lymphoid organs such as the spleen, lymph nodes, mucosal lymphoid tissues, and bone marrow, where it destroys more cells. It also invades and destroys the thymus. The shedding of infected cells from these lymphoid organs enables the virus to reach epithelial tissues and the brain. By triggering lymphocyte and macrophage apoptosis and directly destroying lymphocytes, canine distemper

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virus produces profound immunosuppression. The virus suppresses production of interleukin-1 (IL-1) and IL-2 while stimulating prostaglandin release by macrophages. As a result, lymphocyte responses to mitogens are depressed, immunoglobulin levels fall, and immediate hypersensitivity reactions and skin graft rejection are suppressed. This immunosuppression accounts, in large part, for the clinical signs of canine distemper. For example, many dogs with distemper develop *Pneumocystis carinii* pneumonia. (*P. carinii* is a fungus that occurs in the lungs. It does not cause disease in immunocompetent animals but produces a severe pneumonia in animals with suppressed immune function. Indeed, the development of *Pneumocystis* pneumonia is evidence of a significant immunodeficiency.) If germfree dogs are infected by virulent distemper virus, they develop a relatively mild disease, presumably because secondary infection cannot occur ([Figure 35-1](#)).

Loss of lymphocytes is common in virus infections, since viral survival and persistence may require immune suppression. Thus a lymphopenia occurs in feline panleukopenia, canine parvovirus-2 infection, feline leukemia, and African swine fever. Bovine viral diarrhea virus (BVDV) causes destruction of both B and T cells in the lymph nodes, spleen, thymus, and Peyer's patches. As in canine distemper, surviving B cells fail to make immunoglobulins and respond poorly to mitogens. Viral destruction of the Peyer's patches causes intestinal ulceration and leads to secondary bacterial invasion. Both persistently infected cattle and normal cattle infected with cytopathic BVDV show depressed neutrophil functions, and bacterial clearance from blood is impaired. A related virus, border disease virus, preferentially infects CD8⁺ T cells and interferes with their cytotoxic and immunoregulatory functions.

Herpesviruses are also immunosuppressive. For example, equine herpesvirus-1 causes a drop in T cell numbers and depresses cell-mediated responses in foals. Bovine herpesvirus-1 (BHV-1) also causes a drop in T cells and in the responses to T cell mitogens. Although BHV-1 stimulates bovine alveolar macrophages to express increased amounts of major histocompatibility complex (MHC) class II molecules and promotes antibody-mediated phagocytosis, it also depresses macrophage-mediated cytotoxicity and IL-1 synthesis. Parainfluenza 3 and infectious bovine rhinotracheitis viruses have long been known to interfere with alveolar macrophage function. They inhibit phagosome-lysosome fusion, thus paving the way for secondary infections with *Mannheimia hemolytica* in stressed calves. Porcine reproductive and respiratory syndrome virus in pigs causes destruction of alveolar macrophages and so predisposes affected animals to severe enzootic pneumonia. It also kills dendritic cells—a feature that may account for its ability to persist in pigs for up to 6 months.

The effect of some viruses on the immune system may be relatively complex or anomalous ([Box 35-1](#)). In canine distemper, for instance, lymphocyte responses to phytohemagglutinin are depressed, but allograft rejection may remain normal. In visna, a neurological disease of sheep caused by a retrovirus, cell-mediated immune responses such as graft rejection are suppressed, whereas B cell responses are enhanced (see [Chapter 23](#)). Some leukemia viruses can exert selective depressive effects, so that depression of the immunoglobulin G (IgG) response is greater than that of the IgM response. In equine infectious anemia, the IgG3 response is variably depressed, whereas synthesis of the other immunoglobulin classes remains unaffected. It has been claimed that chickens infected with Marek's disease virus show both enhanced graft-versus-host disease and depressed graft rejection.

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35.2.1 Box 35-1 Viruses That Affect Lymphoid Tissues of Animals

35.2.1.1 Viruses that Destroy Lymphoid Tissues

- Human immunodeficiency virus
- Measles virus

- Simian immunodeficiency virus
- Simian retrovirus, type D
- Feline immunodeficiency virus
- Feline leukemia virus
- Feline panleukopenia virus
- Equine herpesvirus-1
- Canine distemper virus
- African swine fever virus
- Bovine viral diarrhea virus
- Mouse thymus herpesvirus
- Infectious bursal disease virus
- Newcastle disease virus
- Porcine circovirus

35.2.1.2 Viruses that Stimulate Lymphoid Tissue Activity to an Unusual Extent

- Maedi-visna virus
- Aleutian disease virus
- Porcine reproductive and respiratory syndrome virus
- Malignant catarrhal fever virus

35.2.1.3 Viruses That Cause Lymphoid Neoplasia

- Marek's disease virus
- Feline leukemia virus
- Bovine leukemia virus
- Mouse leukemia virus
- Human T cell leukemia virus 1

The results of virus-induced lymphoid tissue destruction are readily seen. Animals are lymphopenic and have reduced lymphocyte responses to mitogens. For example, the responses to phytohemagglutinin are depressed in influenza, measles, canine distemper, Marek's disease, Newcastle disease, feline leukemia, BVDV, and

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lymphocytic choriomeningitis. Destruction of lymphoid tissue may also result in hypogammaglobulinemia or a reduced response to antigens. Thymic atrophy and lymphopenia are common manifestations of many virus infections, and before any primary immunodeficiency syndrome is diagnosed, rigorous steps must be taken to exclude the possibility that it is, in fact, secondary to a virus infection.

35.3 RETROVIRUS INFECTIONS IN PRIMATES

Human immunodeficiency virus (HIV-1) almost certainly originated from a strain of simian immunodeficiency virus (SIV_{cpz}) that normally infects chimpanzees (*Pan troglodytes*) in parts of central Africa. SIV_{cpz} probably jumped to humans and mutated slightly to become HIV-1. It can infect but very rarely causes disease in chimpanzees. Other primates such as pig-tailed macaques (*Macaca nemestrina*) can only be transiently infected with HIV-1 but are not immunosuppressed and remain healthy. A distantly related virus, HIV-2, can cause disease in baboons (*Papio cyanocephalus*) and pig-tailed macaques, but its natural reservoir is the sooty mangabey (*Cercocebus atys*). Rhesus (*Macaca mulatta*) and cynomolgus (*Macaca fascicularis*) macaques may be persistently infected with HIV-2 but do not develop disease. There are several interesting nonprimate models of HIV infection. Rabbits are susceptible to persistent HIV infection but, like macaques, do not develop disease. When reconstituted with human lymphocytes, *scid* mice can harbor HIV, but the virus destroys their T cells very rapidly in a manner unlike the natural disease.

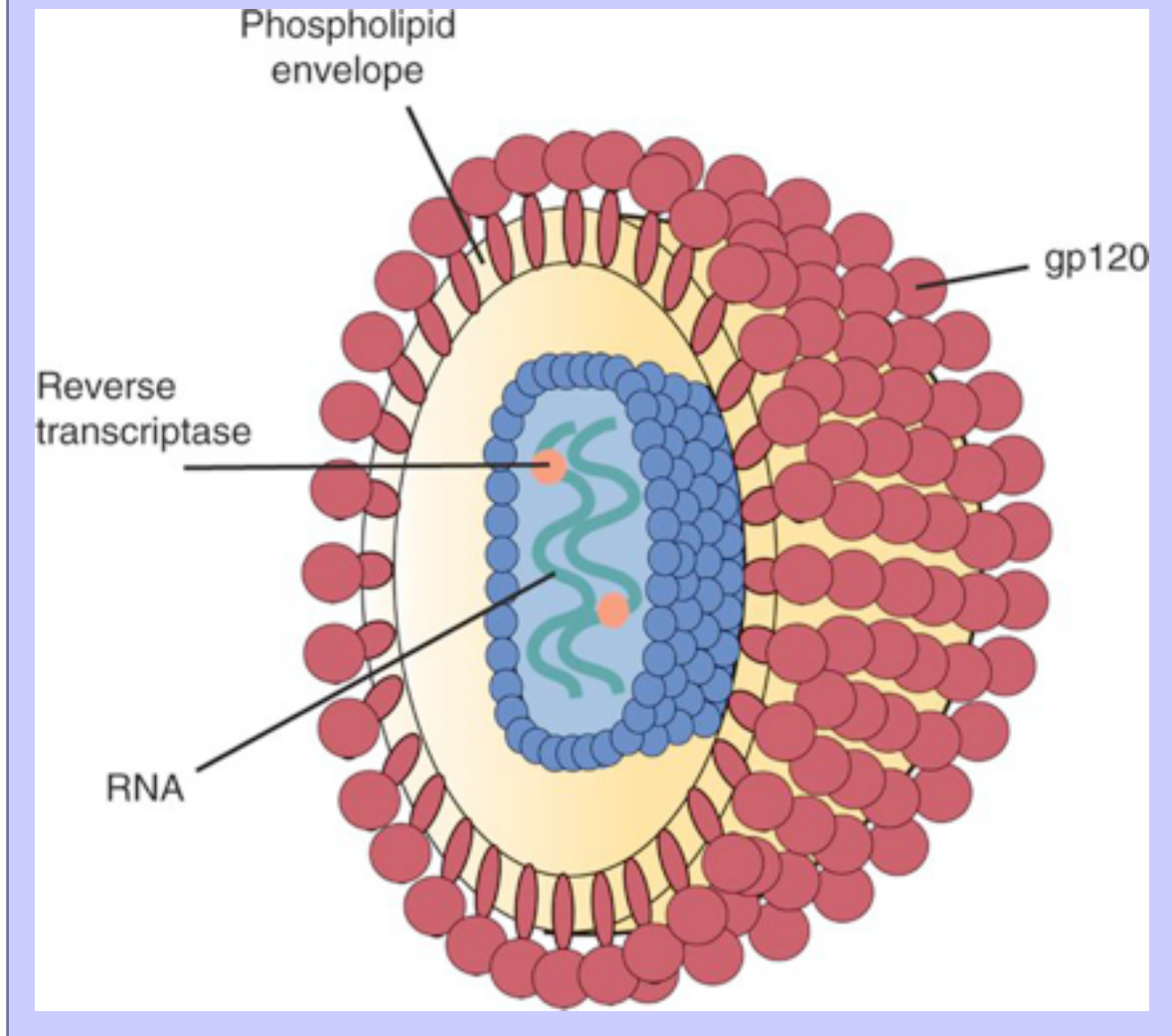
Many different lentiviruses have been isolated from primates, especially African species. These SIVs include SIV_{mac} isolated from a rhesus macaque in a laboratory; SIV_{agm} from an African green monkey; SIV_{sm} from a sooty mangabey; SIV_{mnd} from a mandrill; and, most important, SIV_{cpz} from a chimpanzee. As pointed out previously, SIV_{cpz} is probably the ancestor of HIV-1, whereas SIV_{sm} is the ancestor of HIV-2. All these isolates selectively invade CD4⁺ T cells. When SIV_{mac} infects rhesus macaques and other Asian species, it causes an immunodeficiency syndrome similar to human AIDS. Sexual transmission is suspected. The animals develop lymphadenopathy, severe weight loss, chronic diarrhea, lymphomas, neurological lesions, and opportunistic infections by organisms such as *P. carinii*, *Mycobacterium avium-intracellulare*, *Candida albicans*, and *Cryptosporidium parvum*. The macaques are immunosuppressed as a result of depletion of CD4⁺ T cells and macrophages. About 25% of infected animals do not mount a significant response to SIV and die within 3 to 5 months, with the remainder usually dying 1 to 3 years after infection. Spontaneous recovery does not occur. The other SIVs cause persistent viremia but rarely result in disease in African primates.

35.3.1 Type D Simian Retroviruses

An acquired immunodeficiency syndrome develops in primates infected with one of several endogenous type

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FIGURE 35-2 The structure of a typical retrovirus such as feline leukemia virus or feline lentivirus.



D simian retroviruses (SRVs). These viruses, much more common than the lentiviruses, are transmitted by biting, with inoculation of saliva or blood; vertical transmission rarely occurs. SRVs have a much broader tissue tropism than the SIVs and, in addition to lymphocytes and macrophages, can also infect fibroblasts, epithelial cells, and the central nervous system. The SRVs destroy both B and T cells, leading to death from opportunistic infections. The syndrome is associated with a profound drop in serum IgG and IgM levels and a severe lymphopenia. Monocyte function is unimpaired, but surviving lymphocytes do not respond to mitogens. Affected monkeys are also profoundly neutropenic. On necropsy, the monkeys have a generalized lymphadenopathy, hepatomegaly, and splenomegaly. There is a loss of lymphocytes from the T-dependent areas of the secondary lymphoid organs. B cell areas show an initial hyperplasia of the secondary follicles followed by the loss of these follicles and an absence of plasma cells. These histological changes are very similar to those seen in AIDS in humans. In many cases, normally innocuous agents such as *P. carinii*, *cytomegalovirus*, *C. parvum*, and *C. albicans* cause infection. Some affected monkeys develop tumors such as fibrosarcomas. About one half of the

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infected animals develop neutralizing antibodies and survive the disease. The others die from septicemia or diarrhea with wasting.

35.4 RETROVIRUS INFECTIONS IN CATS

35.4.1 Feline Leukemia

Feline leukemia virus (FeLV) is an oncogenic retrovirus that can cause both proliferative and degenerative diseases in cats ([Figure 35-2](#)). Three naturally occurring viral subgroups are recognized based on the structure of their gp70 protein. FeLV-A is the predominant, naturally transmitted subgroup. It is present in all FeLV-infected cats. The viruses of the other subgroups are only found in association with FeLV-A. When FeLV-A combines with endogenous retroviral sequences (sequences that are stably integrated into the cat genome and are normally never expressed but are genetically transmitted), FeLV-B is formed. FeLV-B is found in about 50% of viremic cats and has a greater propensity than FeLV-A to cause tumors. FeLV-C is found in about 1% to 2% of infected cats and arises from FeLV-A through a mutation in the envelope gene. It is much more suppressive for bone marrow than FeLV-A.

35.4.1.1 FOCMA

In many cases of feline lymphosarcoma, a unique surface protein is expressed on infected cells. This protein is called feline oncornavirus cell membrane antigen (FOCMA). Endogenous retroviral genes within the cat genome code for FOCMA. It is not expressed on normal cells but on cells infected with FeLV or feline sarcoma virus. It was originally believed that the presence of FOCMA on a cell membrane identified the cell as an FeLV-induced tumor cell. Of those cats that fail to make neutralizing antibodies to FeLV and so remain viremic, about 80% develop antitumor activity by making antibodies against FOCMA. A cat that makes antibodies to FOCMA can usually destroy virus-induced tumor cells. Unfortunately, antibodies to FOCMA do not confer protection against the FeLV-induced degenerative diseases, and viremic cats that fail to produce anti-FOCMA antibodies are fully susceptible to all the FeLV syndromes, including lymphosarcoma. Some feline lymphosarcoma cells may express FOCMA in the absence of any evidence of FeLV infection.

35.4.1.2 Transmission

FeLV is shed in secretions, especially saliva and nasal secretions, and is thus transmitted between cats as a result of grooming. On natural exposure to FeLV, about 70% of cats become infected, but the remaining 30% do not. Of the infected cats, about 60% become immune and 40% become viremic. Of viremic cats, 10% cure spontaneously, whereas the remaining 90% remain infected for life. Of these persistently viremic animals, about 15% live normal healthy lives, but the remaining animals die within 3 to 5 years from FeLV disease. Lymphoid tumors develop in 15% to 20% of FeLV-infected cats. Persistently viremic cats have a half-life of 1 year.

35.4.1.3 Pathogenesis

Once FeLV infects a cat, the virus first grows in the lymphoid tissues of the pharynx and tonsils. This is followed by a transient viremia as it spreads throughout the body and infects all the other lymphoid organs. A mild lymphopenia occurs 1 to 2 weeks after infection. This is of variable duration but lasts longer in young cats than in adults. There is also a variable neutropenia. Antibodies develop between 7 and 42 days after the

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onset of infection and the virus is cleared between 28 and 42 days. Virus can be found in the thymus at day 1, in blood between 2 and 145 days, and in lymphoid organs between 3 and 28 days. The presence of both antibodies and virus results in the formation of large amounts of immune complexes and the development of a membranoproliferative glomerulonephritis. Some cats may lose their viremia but remain latently infected. In latently infected animals, the virus persists in the bone marrow, but there is no virus in the blood and virus-neutralizing antibodies are present. Treatment with steroids or culturing bone marrow cells in vitro allows productive reexpression of the virus. Stress (for example, steroid treatment, crowding, or shipping) may cause a recurrence of viremia in 5% to 10% of cats. The presence of neutralizing antibodies does not correlate well with the disease state, and challenging recovered cats may not provoke a secondary antibody response. Prenatal or early infection of kittens with FeLV can also result in a persistent viremia.

35.4.1.4

Tumors

Cats probably have the highest prevalence of lymphoid tumors of any domestic mammal. Most of these lymphoid tumors are caused by FeLV. They include lymphosarcoma, reticulum cell sarcoma, erythroleukemia, and granulocytic leukemias. The lymphosarcoma caused by FeLV is usually a T cell neoplasm, although FeLV grows in cells of many types and is not restricted to lymphoid tissues. Some FeLV lymphomas in the intestine may be of B cell origin. When tumors develop in FeLV-infected cats, not all can be shown to contain the virus. The proportion of positive tumors ranges from 100% of myeloid leukemias to 30% of alimentary lymphomas. In young cats, FeLV-induced tumors are mainly of T cell origin. In older cats they tend to be both T and B cell in origin.

35.4.1.5

Immunosuppression

35.4.1.5.1

T Cell Defects

FeLV develops T cell tropic variants as a result of mutations in their envelope genes. These immunodeficiency-inducing variants can replicate to high numbers in T cells. They enter T cells by binding to two receptors. One receptor is a phosphate transporter protein (Pit1). The second is a novel cell surface protein called FeLIX ("FeLV infectivity X-essory protein"). The lymphopenia in FeLV-infected cats is due to a loss of CD4⁺ T cells. CD8⁺ T cells may also drop in the early stages of the disease, so the CD4/CD8 ratio may remain within normal limits. (The CD4 : CD8 ratio in normal cats ranges from about 0.4 to 3.5, with a median value of about 1.9.) However, as CD8⁺ T cell numbers recover, the CD4 : CD8 ratio may drop. B cell numbers may also be depressed, but this depends on the severity of secondary infections. Kittens infected with FeLV develop a wasting syndrome associated with thymic atrophy and recurrent infections. Depending on the severity of the secondary infections, this may be associated with either lymphoid atrophy or lymphoid hyperplasia. In cats without secondary infection, lymphoid atrophy is associated with loss of cells from the paracortical areas of lymph nodes. The changes in the spleens of these animals are less marked but may result in a reduction in the entire white pulp. As a result of T cell loss, FeLV-infected cats have depressed cell-mediated immunity. This depression is probably due to the effects of p15e, the immunosuppressive envelope protein of the FeLV virus, which is produced in very large quantities by dying cells. p15e suppresses the responses of cats to FOCMA, suppresses lymphocyte mitogen responses, and blocks the responses of T cells to IL-1 and IL-2. As a result, FeLV-infected cats may carry skin allografts for about twice as long as normal cats (24 days as compared with 12). The leukocytes of cats infected with FeLV produce significantly less IL-2 than leukocytes from normal cats. This decline in IL-2 production is especially marked in cats with leukemia or lymphosarcoma arising in the thymus. This immunosuppression

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also predisposes viremic cats to secondary diseases such as feline infectious peritonitis, mycoplasmosis, toxoplasmosis, septicemia, and fungal infections. Bone marrow stem cells are also inhibited by p15e, preventing production of erythroid cells and causing a non-regenerative anemia.

35.4.1.5.2

B Cell Defects

In contrast to the severe T cell dysfunction, B cell activities in FeLV infected cats are only mildly impaired. There may be poor responses to low doses of antigen, as well as reduced IgM production, but serum IgG levels remain normal. Because B cell function and antibody production are relatively normal in chronically infected cats, antibodies to the virus are produced in large quantities. These antibodies combine with circulating virions or soluble proteins to form immune complexes. The immune complexes are deposited in the renal glomeruli and cause severe membranoproliferative glomerulonephritis, leading to hypoproteinemia, edema, uremia, and death. Viral antigens binding to erythrocytes can also cause an antiglobulin-positive hemolytic anemia. Immune complexes also activate the classical complement pathway. As a result, complement will be consumed and FeLV cats may have very low levels of complement. This loss may reduce resistance to tumors since normal cat serum infused into leukemic cats can cause tumor regression.

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35.4.1.6

FeLV-AIDS

During natural FeLV infection a form of the virus may develop that is profoundly immunosuppressive. Called FeLV-AIDS, this causes fatal immunodeficiency in nearly 100% of infected cats. The isolate consists of two virus populations. One, designated 61E, is replication-competent but does not induce immunodeficiency disease by itself. The other, 61C, is replication-defective, but when inoculated together with 61E, it induces a fatal immunodeficiency syndrome. A viral chimera has been constructed that has properties intermediate between those of the two originating strains.

The immunodeficiency syndrome is characterized by progressive weight loss and lymphoid hyperplasia followed by severe lymphoid depletion, chronic diarrhea, and opportunistic infections. The onset of clinical disease is preceded by the accumulation of unintegrated viral DNA in the bone marrow and by an early and drastic drop in CD4⁺ T cells, whereas CD8⁺ T cell and B cell numbers remain normal. Analysis has shown that the immunodeficiency is associated with mutations in a 34 amino acid sequence at the C-terminus of viral gp70. The mutation changes the conformation of the surface glycoprotein, which affects the ability of the virus to bind to cell receptors and block infection by additional virions leading to subsequent cell killing. The defect in FeLV-AIDS is an inability to mount antibody responses, although in vitro B cell function appears to be normal. An inability to respond to T-independent antigens precedes the loss of CD4⁺ T cells. Thus, as early as 9 weeks after infection, the CD4⁺ T cells produce lower levels of B cell stimulatory cytokines.

35.4.1.7

Immunity

About 40% of cats infected with FeLV do not mount an adequate immune response against the virus and become persistently infected. Persistently infected cats remain viremic. The remaining 60% of infected cats mount a strong immune response. These cats develop virus-neutralizing antibodies to the major envelope glycoprotein, gp70. Immune cats also develop virus-specific cytotoxic T cells to viral gag/pro antigens. These prevent the virus from invading cells, and these cats become strongly immune. Antibodies against antigens other than gp70 may also play a role in immunity.

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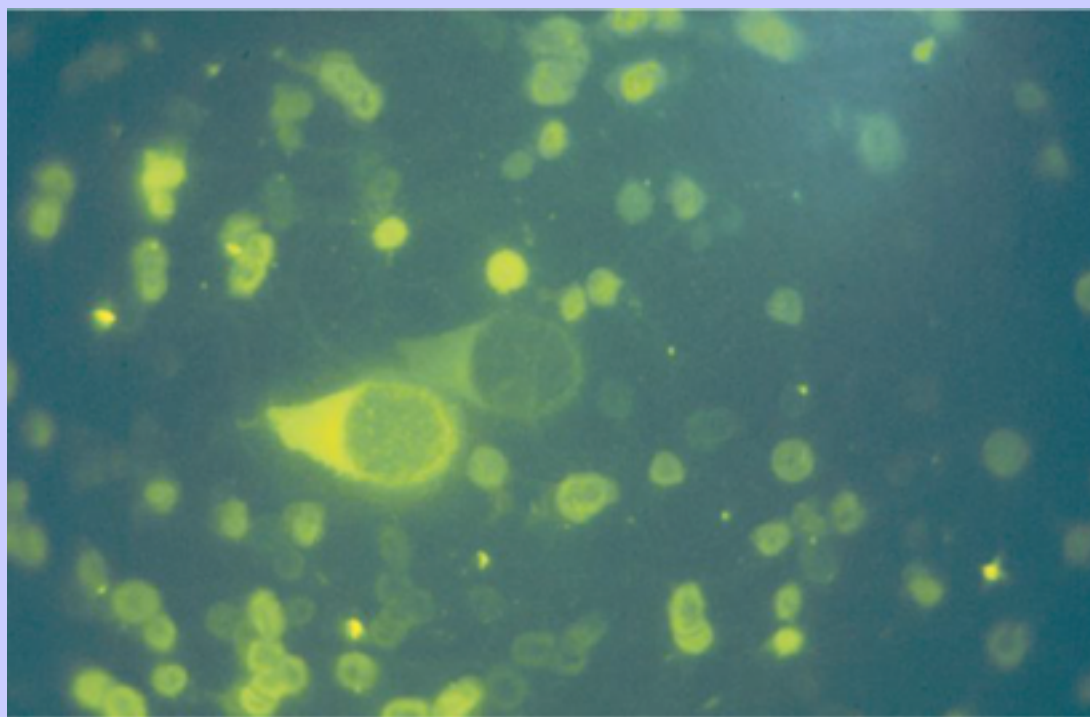
Four types of effective vaccines have been developed against FeLV. One type contains supernatant fluid from a cell line persistently infected with FeLV. This fluid contains several of the major protein antigens of FeLV. The second type of FeLV vaccine consists of inactivated whole virions, which are usually administered with a powerful adjuvant. The third type of FeLV vaccine contains pure gp70 obtained from recombinant *Escherichia coli*. Resistance to FeLV is associated with a response to gp70, but other virion antigens are required for maximal protection. The most recent type of FeLV vaccine is a canarypox-vectored recombinant product. Widespread vaccination has significantly reduced the prevalence of this disease in the United States.

35.4.1.8

Diagnosis

FeLV viremia may be detected by an enzyme-linked immunosorbent assay (ELISA), by the membrane filter technique, or by rapid immunochromatography on a blood or serum sample. A direct immunofluorescent test on a buffy coat smear using antibodies to group-specific antigen can detect cell-associated antigen ([Figure 35-3](#)). Some of these tests may detect infection before the development of viremia, since soluble virus antigens are shed into the bloodstream. Alternative testing methods include testing saliva or tears using material collected on a swab or filter paper strips. Polymerase chain reaction (PCR)-based assays may be used to detect viral nucleic acid.

FIGURE 35-3 A positive indirect immunofluorescence assay for feline leukemia virus in a peripheral blood smear. (Courtesy Dr. F.C. Heck.)



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35.4.2 Feline Immunodeficiency Virus

Feline immunodeficiency virus (FIV) was originally isolated from cats with clinical immunodeficiency. The virus is an enveloped, single-stranded RNA virus belonging to the lentivirus subgroup of retroviruses. It is differentiated from FeLV (a gamma retrovirus) by the biochemical requirements of its reverse transcriptase. (FIV reverse transcriptase requires magnesium, whereas FeLV requires manganese.) FIV is related to HIV, the cause of AIDS ([Figure 35-4](#)). FeLV and FIV are distinctly different viruses, and antibodies made against one do not react with the other. Nevertheless, approximately 12% to 33% of FIV-infected cats may also be infected with FeLV, an especially potent immunosuppressive mixture. At least five different genetic subtypes of FIV have been identified. Subtype variations may account for differences in pathogenicity, tissue tropism, and clinical disease.

35.4.2.1 Transmission

FIV is spread by territorial free-roaming male cats through aggressive biting. As a result, it occurs predominantly in old male cats that spend a lot of time outdoors. Oral exposure is a potential route of infection in suckling kittens, and chronically infected queens transmit viruses to more than half their kittens in utero. Noninfected kittens from chronically infected queens show reduced neonatal viability. FIV can also be sexually transmitted. In households where cats live together nonaggressively, sharing food or water bowls and undergoing mutual grooming, the infection is poorly spread. In the United States, 1% to 3% of normal healthy cats and 10% to 15% of chronically ill cats are infected with FIV. In some countries, such as Japan, the infection rate may be as high as 44%.

35.4.2.2 Pathogenesis

Experimental infection of cats with FIV is characterized by four distinct clinical stages. The acute stage lasts for several weeks. Infected cats develop a fever about 3 to 10 weeks after exposure to FIV. The virus is carried to local lymph nodes, where it replicates in T cells. It then spreads to other lymph nodes throughout the body. FIV can be isolated from infected cats as early as 10 to 14 days after infection. Viremia increases until day 21, peaks again at 7 to 8 weeks, and then declines. Disease severity varies from no clinical signs to generalized lymphadenopathy and lymphoid hyperplasia. Cats can develop fever, anorexia, dehydration, and diarrhea with mild pneumonitis, conjunctivitis, and nephritis. They may develop a mild lymphopenia and a severe neutropenia at this time. The lymphopenia is due to a loss of $CD4^+$ T cells. Cats rarely die at this stage unless they are also infected with FeLV, in which case they die of a panleukopenia. Antibodies to FIV develop 2 to 6 weeks after infection and persist throughout infection. Most cats recover from this stage and appear normal.

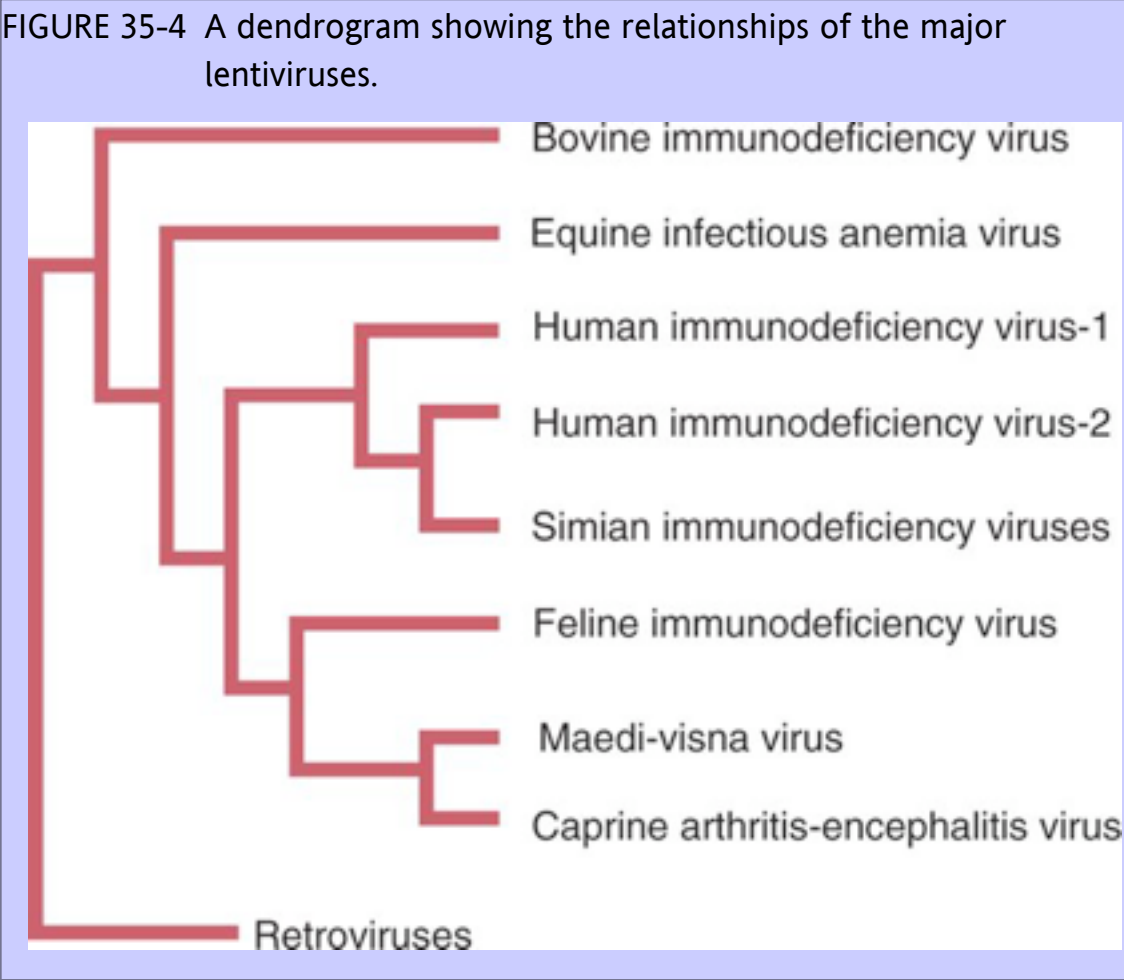
The asymptomatic, or latent, stage may last as long as 10 years and is longer in young than in older cats. Cats remain clinically healthy during this stage, but there is a progressive drop in their $CD4^+$ T cell numbers. Their lymph nodes show gradual hypoplasia, leading to aplasia. Cats may also develop bone marrow suppression, including leukopenia and anemia. Thus this stage is marked by progressive impairment of immune function, but it may be many years before severe immunodeficiency and AIDS-like signs develop.

The gradual onset of progressive generalized lymphadenopathy marks the third stage of the disease. This lasts for months to years and is associated with vague signs of ill health, such as recurrent fever, inappetence, weight loss, chronic stomatitis, arthritis, and behavioral abnormalities. Lymph nodes develop follicular hyperplasia. As a result of the growing immunodeficiency, cats may develop secondary but not opportunistic

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infections. These are mainly bacterial infections affecting the oral cavity, skin, and digestive tract. Cats will show some weight loss (less than 20%), anemia, lymphopenia, and neutropenia.

The final stage is a severe AIDS-like disease that lasts for a few months until the cat dies. Lymphoid tissue shows follicular involution. Cats show a weight loss of greater than 20%. Because of their severe immunodeficiency, opportunistic infections develop. These can include feline herpesvirus type 1, rodent poxviruses, vaccine-induced rabies, FeLV, staphylococcal infections, anaerobic infections, tuberculosis (*M. avium-intracellulare*), *Cryptococcus*, *toxoplasmosis*, *mange*, *lungworms*, and *heartworms*. The animals have anemia, lymphopenia, and neutropenia. Malig



nancies and ocular and neurological disease also occur. In naturally infected cats, clinical findings are highly variable because of the great variety of potential secondary infections. They can include chronic fever, oral cavity disease (periodontitis/gingivitis/stomatitis) leading to inappetence or pain on eating, chronic upper respiratory tract disease, chronic enteritis leading to persistent diarrhea, and conjunctivitis. Some cats may experience cystitis, chronic skin disease, fever, anorexia, lethargy, abortion or reproductive problems, vomiting, anemia, leukopenia, lymphosarcoma, and myeloproliferative disorders. Neurological signs have been described in FIV-infected cats, and the virus has been shown to infect the central nervous system. Half of FIV-infected cats have neurological dysfunction, as demonstrated by abnormal behavior, convulsions, ataxia, paralysis, and nystagmus. FIV is associated with demyelination in the dorsal columns of the spinal cord,

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vacuolization of the myelin sheaths in the spinal nerve roots, and perivascular and perineuronal mononuclear cell infiltration. Ocular lesions, especially anterior uveitis, conjunctivitis, and glaucoma have also been noted.

35.4.2.3

Immunosuppression

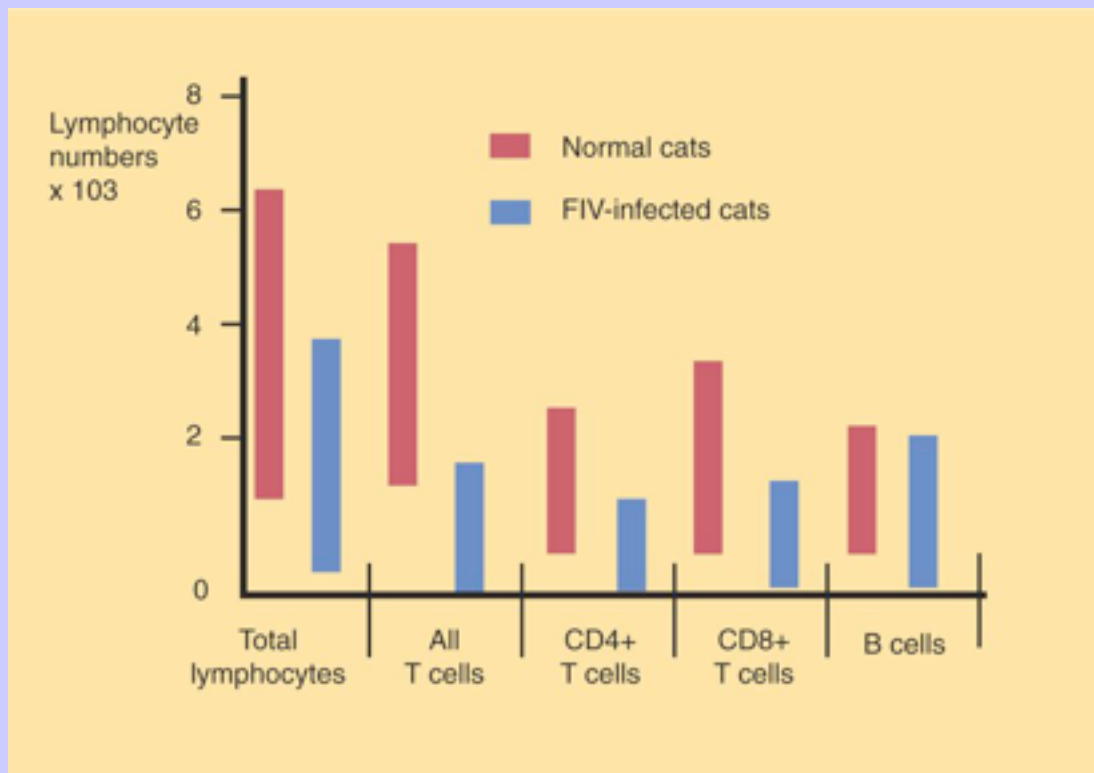
FIV can replicate in CD4⁺ and CD8⁺ T cells, B cells, megakaryocytes, neuronal cells, and macrophages. Some strains replicate well only in lymphocytes, whereas other strains replicate well in both lymphocytes and macrophages. Some FIV strains can also grow in fibroblasts in vitro. Primary targets of FIV infection are the lymphocytes. However, as infection persists, the virus increasingly affects macrophages. In clinically ill cats with a high viral load, macrophages are the major sites of viral replication. FIV-infected cats have fewer neutrophils, a lower proportion of T cells, and a higher proportion of B cells compared with uninfected animals.

FIV binds specifically to CD134 expressed on a subset of CD4⁺ T cells. This binding, in conjunction with binding to the α -chemokine receptor CXCR4 (CD184), is required for FIV to infect a cell. CD134 glycoprotein is upregulated on activated CD4⁺ T cells but not on activated CD8⁺ T cells.

Most naturally infected cats have a critical loss of CD4⁺ T cells ([Figure 35-5](#)). This loss is a result of destruction of infected cells, decreased production, and premature apoptosis. The surviving CD4 cells may show reduced responses to mitogens. FIV cats may show a shift away from a Th1 cytokine production pattern. They may also show an increase in CD8⁺ T cells. As a result, the CD4 : CD8 ratio of FIV-infected cats may drop from a normal value of about two to less than one. FIV may also activate CD4⁺CD25⁺ T_{reg} cells, which would further contribute to the immunosuppressive effect.

The lymphopenia that develops in both FeLV and FIV infections is due to a loss of T cells. CD4⁺ T cells are depressed in both, but the depression is much greater in FIV-infected animals than in FeLV-infected animals. FIV-infected cats show a rapid drop in T cell numbers, whereas B cells are unaffected. Their CD8⁺ T cells recover, but their CD4⁺ T cells fail to do so. Within 6 months of FIV infection, there is a measurable drop in CD4⁺ T cells and in the lymphocyte response

FIGURE 35-5 The numbers of cells in different lymphocyte populations (pan T, CD4, CD8, B cells) for 11 normal cats and 11 cats infected with feline immunodeficiency virus. (From Novotney C, English RV, Housman J, et al: *AIDS* 4:1213-1218, 1990.)



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to pokeweed mitogen (PWM), a B cell mitogen. (The B cell response to PWM requires functional CD4⁺ T cells.) The response to thymus-dependent and thymus-independent antigens remains unchanged. By 2 to 3 years after the onset of infection, however, the drop in CD4⁺ T cells continues, and the response to the T cell mitogen, Con A, becomes depressed. The response to thymus-dependent antigens is profoundly depressed by this stage, but the response to thymus-independent antigens is normal. Other changes that occur in FIV-infected cats include depressed responses to phytohemagglutinin and lipopolysaccharide, reduced production of IL-2, and depressed responses to IL-2. Affected cats may upregulate IL-10 transcription, which may contribute to their immunodeficiency. FIV-infected cats may have normal numbers of CD8⁺ T cells and B cells and normal levels of IgM and IgA. More than 25% of FIV-infected cats may be hypergammaglobulinemic as a result of polyclonal B cell activation 6 to 8 weeks after infection. Despite this, the response of FIV-infected cats to antigens such as diphtheria toxoid may be depressed. Affected cats may have high levels of immune complexes in their serum and deposited in renal glomeruli.

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35.4.2.4 Immunity and Diagnosis

Clinical symptoms are not sufficient to reliably diagnose FIV infection. The infection is therefore diagnosed by testing for antibodies in serum by ELISA or immunochromatography and should be confirmed by Western blotting or PCR. Antibodies appear as early as 2 weeks after infection, and most cats are positive by 60 days. These antibodies persist for the life of the animal. In the terminal stages antibodies may fall to undetectable levels. Maternal antibodies persist in most kittens born to FIV-positive queens for the first 8 to 12 weeks of life, regardless of whether the kitten is infected. Some may remain seropositive up to 16 weeks. These antibodies afford protection since passive immunization is effective; kittens that receive high levels of antibodies from vaccinated or infected queens are protected.

Once infected with FIV, a cat will remain infected for life. However, viral regression may occur in kittens vertically infected by their mothers. At 3 to 4 months of age, these kittens may lose detectable antibody and virus in their blood, although the virus persists at low levels in bone marrow and lymph nodes. The presence of very low levels of viruses in an animal can be detected by PCR.

Envelope glycoproteins do stimulate strong cell-mediated and humoral immunity in cats. Good results have been obtained using inactivated whole FIV and certain DNA vaccines. An adjuvanted, inactivated vaccine against FIV clades A and D is commercially available. It is claimed to give protection against clade B as well.

35.4.2.5 Treatment

Treatment of FIV infection is symptomatic and can involve the use of antibiotics to control bacterial infections, fluid therapy, and possibly dietary supplements. AZT (zidovudine, azidothymidine) is the only drug known to have an antiviral effect in clinically affected cats. It appears to improve the health of affected cats and increases both their quality of life and survival. Unfortunately, AZT-resistant strains of FIV may develop, and the drug seems to be of limited benefit in clinically ill cats. Encouraging results have also been obtained by the use of bone marrow allografts in association with antiviral therapy.

35.5 RETROVIRUS INFECTIONS IN CATTLE

35.5.1 Bovine Immunodeficiency Virus

Bovine immunodeficiency virus (BIV) is a lentivirus originally isolated from a cow with lymphosarcoma. The BIV-infected animal showed lymph node hyperplasia, lymphocytosis, central nervous system lesions, loss of weight, and weakness. When used to infect calves, BIV shows limited pathogenicity. The animals develop transient lymphocytosis, lymphadenopathy, and a nonsuppurative meningoencephalitis. BIV infection may also cause minor changes in the response of lymphocytes to mitogens and may suppress some neutrophil functions, such as antibody-dependent cell-mediated cytotoxicity, several months after infection. BIV can also infect sheep. In this species, infection is associated with an increase in CD2⁺ and CD4⁺ T cells, as well as in the CD4 : CD8 ratio between 6 and 8 months after inoculation. The sheep showed no signs of illness by 1 year after inoculation and appeared to have normal immune function. BIV can also cause a chronic infection of rabbits, leading to splenomegaly and lymphadenopathy.

Jembrana disease is a lentivirus infection that occurs in Balinese cattle (*Bos javaensis*). It causes intense lymphoproliferation in the lymph nodes and spleen in these cattle. Animals that recover are completely immune.

35.6 RETROVIRUS INFECTIONS IN DOGS

Several different retroviruses have been isolated from dogs. Some have been claimed to be lentiviruses, although the existence of a “canine immunodeficiency virus” has not yet been established. For example, a lentivirus has been isolated from the mononuclear cells of a leukemic German Shepherd dog in Israel. This virus does not appear to be closely related to the other major lentiviruses. On inoculation into newborn beagles, it caused pronounced lymphadenopathy. It is anticipated, however, that several years would pass before major disease symptoms presented themselves.

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Another retrovirus has been isolated from a dog with a severe acquired immunodeficiency syndrome in the United States. This animal had anemia, neutropenia, lymphopenia, and thrombocytopenia, as well as depressed humoral and T cell-mediated immune responses. On necropsy the dog showed depletion of lymphoid organs and bone marrow hypoplasia. Yet it also had plasma cell infiltrates in many organs, as well as multiple secondary infections. The retrovirus isolated from this animal was of the C-type, and it possessed a gene related to the polymerase gene of bovine leukemia virus. It is also interesting to note that this animal had received multiple blood transfusions, a route of infection well recognized for HIV.

A lentivirus has also been isolated from a dog with hemorrhagic gastroenteritis. This animal showed a lymphopenia and agammaglobulinemia with lymphoid and bone marrow hypoplasia. The virus could grow in canine lymphocytes and thymocytes; it had a magnesium-dependent reverse transcriptase. The virus was present in bone marrow, intestine, and lymph nodes. It caused reduced synthesis of IL-2, reduced their responsiveness to IL-2, and was cytotoxic for lymphocytes.

35.7 CIRCOVIRUS INFECTIONS

Circoviruses are small, nonenveloped DNA viruses that have a propensity to damage lymphoid tissues. They include the chicken anemia agent, which infects hemocytoblasts in the bone marrow and precursor T cells in the thymus, beak-and-feather disease virus, which can cause lymphoid atrophy in psittacine birds, and porcine circovirus-2 (PCV2), which may cause postweaning multisystemic wasting syndrome (PMWS). PMWS is an acquired immunodeficiency syndrome of piglets characterized by wasting, lymphadenopathy, and respiratory disease with occasional pallor, jaundice, and diarrhea. Some affected piglets have a profound lymphocyte depletion, initially involving CD4⁺, CD8⁺, and double-positive T cells. T cell areas in tonsils and lymph nodes are depleted, and there is an absence of follicles in the cortex. IgM⁺ B cells are also reduced in more chronic cases. Lymphoid depletion is directly related to viral load in lymphoid organs. Piglets suffer from a variety of secondary and opportunistic infections. While PCV2 is the most likely causative agent, it has proved difficult to reproduce the disease consistently, and other factors are clearly required. These include environmental factors, other infectious agents, and possibly immune stimulation.

35.8 JUVENILE LLAMA IMMUNODEFICIENCY SYNDROME

A severe immunodeficiency syndrome affecting young llamas has been recognized. It has not been reported in other South American camelids. The disease is not due to failure of passive transfer, since the median age of onset is approximately 1 year (range 2 to 30 months). Most affected animals are clinically normal and grow well until weaning. Initial signs include failure to grow, weight loss, and repeated multiple opportunistic infections with a variety of bacteria, fungal, and protozoan organisms. Respiratory tract infections are common. *P. carinii* infection has been recorded in some animals. The animals have low to low-normal lymphocyte numbers. They have

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depressed lymphocyte responses to mitogens such as phytohemagglutinin, concanavalin A, and streptococcal protein A. Lymph node biopsies show marked depletion of T cells in the paracortical areas, and the primary follicles in the B cell areas appear small and lack germinal centers. The animals also have low serum IgG levels and respond very poorly to *Clostridium perfringens* vaccines. Thus both T and B cell responses are depressed. The cause of this syndrome is unknown. Some investigators have detected reverse transcriptase activity in tissues and seen particles on electron microscopy that are compatible with a retrovirus infection. Nevertheless, the consistent occurrence of this disease in young llamas suggests that it may be inherited. Although treatment is supportive, the long-term prognosis for these animals is poor.

35.9 OTHER CAUSES OF SECONDARY IMMUNODEFICIENCY

35.9.1 Microbial and Parasite Infections

Immunosuppression generally accompanies infestation with *Toxoplasma* or trypanosomes, helminths such as *Trichinella spiralis*, arthropods such as *Demodex*, and bacteria such as *Mannheimia hemolytica*, the actinobacilli, and some streptococci.

35.9.2 Toxin-Induced Immunosuppression

Many environmental toxins such as polychlorinated biphenyls, polybrominated biphenyls, dieldrin, iodine, lead, cadmium, methyl mercury, and DDT are immunosuppressive. CdCl₂ and HgCl₂ both inhibit phagocytosis by bovine leukocytes at very low concentrations. Higher concentrations are required to inhibit natural killer (NK) cell function and cell proliferation. Mycotoxins may be important immunosuppressants in cattle or poultry fed moldy grain. These include the T-2 toxin from *Fusarium*, which depresses the response of calf lymphocytes to mitogens and decreases the chemotactic migration of neutrophils. T-2 toxin also reduces IgM, IgA, and C3 levels in cattle. Aflatoxins increase the susceptibility of chickens to *Salmonella* as a result of depressed phagocytic activity. They depress piglet growth and reduced immune responses to Mycoplasma. Fumonisin B1 inhibits division of both T and B cells in piglets, increases interferon- γ (IFN- γ) production while suppressing IL-4 production, and increases susceptibility to *E. coli* infections. Ochratoxins and trichothecenes are also immunosuppressive in pigs and birds. Toxin-induced immunosuppression may be especially important in wild carnivores situated at the top of the food chain. A good example of this is seen in seals feeding on environmentally contaminated fish. These animals show depressed responses to vaccines, impaired mitogenic responses, lowered delayed hypersensitivity responses, and reduced NK cell numbers. This immunosuppression may decrease their resistance to phocine morbillivirus.

35.9.3 Malnutrition and Immunity

It has long been recognized that famine and disease are closely associated, and we tend to assume that malnutrition leads to increased susceptibility to infection. This is not necessarily true, however, because the effects of malnutrition on immune functions are complex. For example, malnutrition can include not only deficiencies but also excesses or imbalances of individual nutrients.

In general, severe nutritional deficiencies reduce T cell function and therefore impair cell-mediated responses, at the same time sparing B cell function and humoral immunity. Thus starvation rapidly induces thymic atrophy and a reduction in the level of thymic hormones. The number of circulating T cells drops, and cells are lost from the T cell areas of secondary lymphoid tissue. Delayed hypersensitivity reactions are reduced, allograft rejection

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is delayed, and interferon production is impaired. Some studies have suggested that protein starvation selectively suppresses Th2 responses such as IL-4 and IgE production, leading to increased susceptibility to parasite invasion.

This starvation-induced immunosuppression is likely mediated through the cytokine leptin. Leptin is a cytokine produced by fat cells (adipocytes). The levels of leptin in blood are proportional to body fat mass. The fatter an individual, the more leptin is produced. Conversely, when an animal starves, adipose tissue shrinks and leptin levels drop. This serves as a signal to the host that energy must be conserved. Leptin is an appetite suppressant and a stimulator of immune function. It has potent proinflammatory activities. Leptin upregulates macrophage phagocytosis and promotes tumor necrosis factor- α (TNF- α) and IL-6 production. It enhances NK cell activity, promotes T cell production, and enhances Th1 activities rather than Th2 activities. Mice deficient in leptin are resistant to autoimmune disease. In humans there is an association between obesity, high leptin levels, and the development of autoimmune diseases such as rheumatoid arthritis. Low levels of leptin, as occurs in starvation, suppress macrophage functions, reduce inflammatory responses, and produce a shift from Th1 to Th2 responses. By suppressing the production of IFN- γ by Th1 cells, leptin provides a mechanism by which metabolically expensive immune functions can be suppressed if food supplies are low.

Severe starvation has little effect on B cell functions. The B cell areas in lymphoid tissues and the number of circulating B cells remain unchanged. Serum immunoglobulins of all classes may remain normal or even rise. Secretory IgA levels commonly drop, but secretory IgE may rise, suggesting abnormal immunoregulation. Starvation will, however, result in depressed complement levels and impairment of neutrophil and macrophage chemotaxis, the respiratory burst, release of lysosomal enzymes, and microbicidal activity.

Several trace elements and vitamins are required for optimal functioning of the immune system. The most important trace elements are zinc, copper, selenium, and iron. Deficiencies of any of these are immunosuppressive. Zinc is especially critical for the proper functioning of the immune system. Zinc deficiency is associated with lymphoid atrophy and delayed cell-mediated responses such as allograft rejection. Zinc-deficient pigs have reduced thymus weight, depressed cytotoxic T cell activity, depressed B cell activity, and depressed NK cell activity. They show decreased antibody production to T-dependent antigens. If pregnant animals are deprived of zinc, their offspring are immunosuppressed. Phagocytic cells from zinc-deficient animals show reduced chemotaxis and microbial ingestion. Mild zinc supplementation may promote immune responses. Copper deficiencies are also immunosuppressive. Thus a copper deficiency reduces neutrophil numbers and function by depressing superoxide production. It also reduces lymphocyte responsiveness to mitogens, reduces T, B, and NK cell numbers, and enhances mast cell histamine release. Selenium deficiency depresses the function of most immune cells, reducing neutrophil activity, T and NK cell responses, and IgM production. Supplementation with selenium upregulates the expression of the IL-2R and prevents oxidative damage to immune cells. Iron deficiency is immunosuppressive for cell-mediated responses. However, the effects of this on resistance to infection may be complex, since many pathogens require iron to replicate. A magnesium deficiency suppresses immunoglobulin levels.

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In general, the antioxidant vitamins A and E are most important for proper immune function. Thus deficiencies of vitamin A reduce lymphocyte proliferation, NK cell activity, and cytokine and immunoglobulin production. Vitamin E is a major antioxidant in cell membranes and is therefore important in regulating the oxidants produced by phagocytic cells. Vitamin E deficiency depresses immunoglobulin levels through its effects on regulatory T cells and results in decreased lymphocyte responses to mitogens. Animals deficient in vitamin E also show reduced IL-2 and transferrin receptor expression and depressed phagocytic function. Vitamin E is one of the few vitamins where supplementation has been shown to enhance immune responses and disease resistance. Other vitamins essential for proper immune function include vitamin B₁₂ and folic acid required for cell-

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mediated immune responses. Some B vitamins are required to maintain immunoglobulin levels, while vitamin D is required for proper macrophage development.

The importance of vitamin E for proper function-ing of the immune system is seen in an inbred population of donkeys whose foals were dying from overwhelming bacterial infections at 3 to 5 months of age. Investigations revealed that these foals were agammaglobulinemic but that they also lacked detectable vitamin E in their serum. Vitamin E supplementation by injection caused an immediate clinical improvement in an affected foal, and within 2 months immunoglobulin levels were within normal limits. It was suggested that affected foals lacked a vitamin E transfer protein, which prevented them from absorbing vitamin E from their feed. All subsequent foals in this herd received supplemental vitamin E and remained healthy.

When an intracellular bacterium such as *Mycobacterium tuberculosis* interacts with toll-like receptor 1 (TLR1) or TLR2 on the surface of macrophages, it upregulates many different genes and enhances their antimicrobial activity. In mice, this is mainly mediated by nitric oxide. In humans, however, nitric oxide is not elevated and other mechanisms are involved. One gene activated by TLR1/2 signaling in humans is that coding for the vitamin D receptor. This receptor is upregulated on activated macrophages. Binding of vitamin D to its receptor in turn upregulates expression of the gene for the antibacterial peptide cathelicidin. Cathelicidin, in turn, can kill intracellular *M. tuberculosis*. It is no coincidence therefore that resistance to tuberculosis is directly related to serum vitamin D levels and that humans with a deficiency of vitamin D show significantly decreased resistance to this infection. It is unclear whether similar mechanisms operate in domestic mammals.

Taurine deficiencies in cats can result in a neutropenia, although mononuclear cell numbers may rise. The neutrophils of taurine-deficient cats show decreased respiratory burst activity and phagocytosis. Although these cats may show a hypergammaglobulinemia, there is regression of follicular centers, suggesting a loss of B cell activity.

The effects of malnutrition may be reflected in altered resistance to infectious diseases. Because bacteria can readily survive and multiply in body tissues despite malnutrition of the host, starvation commonly increases the severity of bacterial infections such as pneumonia. Viruses, in contrast, usually require healthy host cells in which to grow. Malnutrition, by rendering host cells unhealthy, may therefore increase resistance to viruses. Overnutrition can also influence susceptibility to viruses. For example, overfed dogs show an increased susceptibility to canine distemper and canine adenovirus 1. Severe obesity and overeating are functionally immunosuppressive, although it is unclear what mechanisms are involved. Indeed, it is clear that in other situations, the presence of fat cells promotes immunity through release of leptin.

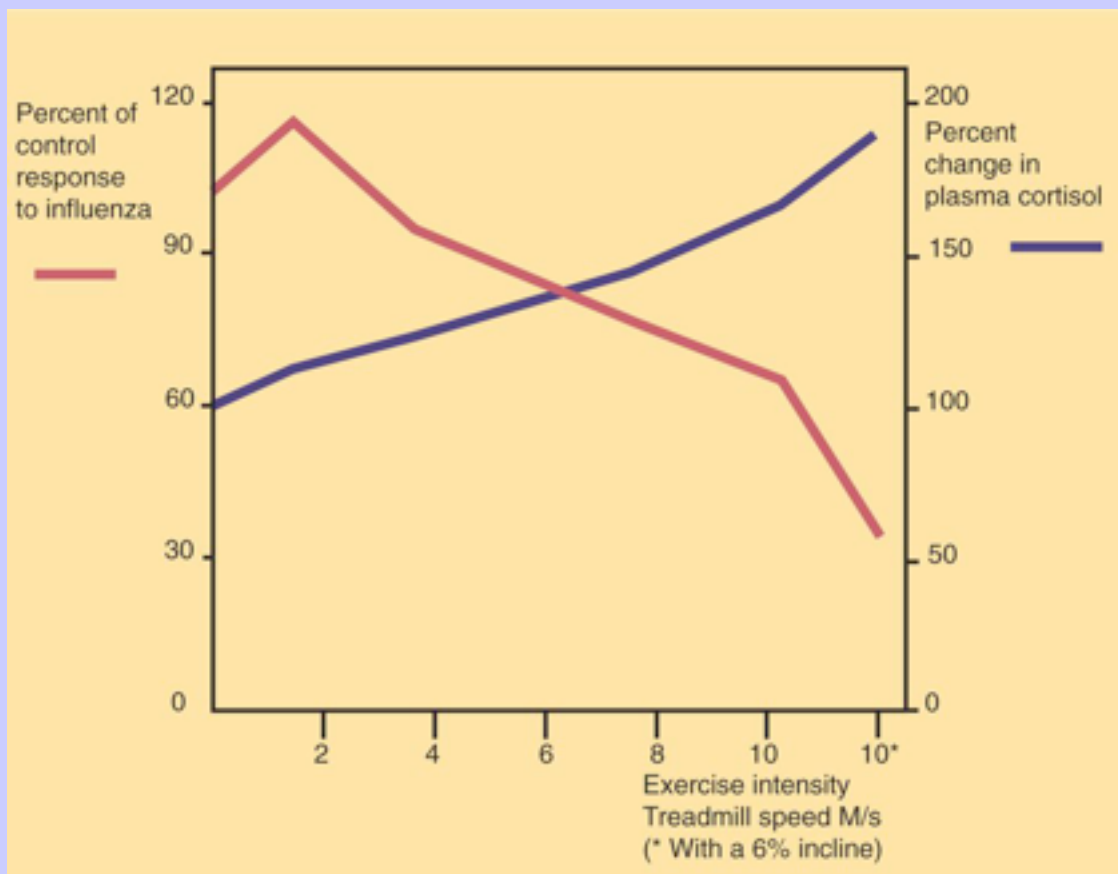
35.9.4

Exercise and Immunity

Regular moderate exercise boosts immune function. Increased antibody responses are seen in mice that get moderate exercise as compared with unexercised control mice. Exercise raises blood neutrophil levels, enhances NK cell activity, promotes lymphocyte responses to mitogens, and increases blood levels of IL-1, IL-6, and TNF- α . As a result, regularly exercised mice show a delay in tumor growth after administration of syngeneic tumor cells. On the other hand, strenuous exercise is stressful and enhances susceptibility to infectious disease. Thus, although mild exercise is good for immune function, high-intensity exercise, prolonged exhaustive exercise, or overtraining may induce a functional immunodeficiency. A decreased proliferative response of blood lymphocytes can be found in horses for up to 16 hours after a race. It is also clear that acute exercise in the unfit animal can be especially stressful. Unfit horses subjected to acute exercise showed significantly raised steroid levels, which corresponded to reduced proliferation of their lymphocytes to mitogens and influenza

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FIGURE 35-6 Although a moderate amount of exercise is good for the immune system, excessive exercise causes severe stress that can be immunosuppressive. In this example, six thoroughbred horses were subjected to a treadmill-based exercise challenge of various intensities (speed and incline). Blood samples were assayed for plasma cortisol levels by radioimmunoassay, and influenza virus–specific lymphocyte proliferation was assayed by thymidine incorporation. A clear relationship exists between exercise intensity, the stress response, and immune responsiveness. (From data kindly provided by Drs. S.G. Kamerling, P.A. Melrose, D.D. French, and D.W. Horohov.)



virus antigens and reduced neutrophil chemotactic responsiveness and chemiluminescence (a measure of respiratory burst activity) (Figure 35-6). These animals show a decline in the CD4 : CD8 ratio, as well as a decline in both the number and activity of NK cells. The age of an animal can moderate the effect of exercise on immune responses. Thus strenuous exercise significantly reduces lymphocyte proliferative responses in young horses yet has much less effect on older animals. This resistance of older horses to exercise-induced

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immunosuppression is probably a result of a reduced steroid production in the older animals. Bulls subjected to stressful exercise become more susceptible to experimental *Mannheimia pneumonia*.

Transportation stress is well recognized as predisposing to the development of respiratory disease in horses. A major reason for the impairment of respiratory defenses in transported horses is prolonged head elevation. With the head held high, mucociliary clearance is significantly reduced. Over time, this elevation permits the accumulation of bacteria, particulates, and inflammatory exudates in the trachea. After 24 hours of head elevation, significant pulmonary inflammation develops. It takes about 12 hours of free head movement for this inflammation to decrease to normal levels.

35.9.5

Posttraumatic Immune Deficiency

Severely traumatized or burned animals commonly die of sepsis as a result of an immunodeficiency. This is mostly due to the production of large amounts of IL-10 and other immunosuppressive cytokines by macrophages. Corticosteroids, prostaglandins from damaged tissues, and a small protein called suppressive active peptide, which appears in serum following a burn, all have immunosuppressive properties. The deficiency occurs within minutes or hours and recovers as wounds heal. It affects T cell, macrophage, and neutrophil function, but B cell function appears to be normal. As a result, delayed hypersensitivity reactions, allograft rejection, and T-dependent antibody responses are all impaired. IL-2 and IL-2R production are reduced. CD8⁺ cells are increased in injured individuals, suggesting that regulatory cell function may be enhanced. Macrophages lose antigen-presenting ability as they express decreased levels of MHC class II molecules. Neutrophil and macrophage phagocytosis and respiratory burst activities are both impaired. Although surgery can result in some suppression of lymphocyte responses to mitogens, evidence suggests that routine surgery has no significant effect on the response of healthy animals to vaccination.

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Table 35-1 Changes in the Immune System Associated with Ageing

Cell Type	Cell Function	Change with Increased Age
Neutrophils	Respiratory burst	Reduced
	Apoptosis	Increased
Macrophages	Respiratory burst	Reduced
	NO production	Reduced
	IL-6 production	Reduced
Dendritic cells	B cell stimulation	Reduced
	Numbers	Reduced
NK cells	Numbers	Increased
	Killing activity	Reduced
NKT cells	Numbers	Increased

35.9.6

Age and Immunity

Innate, cell-mediated, and humoral immune responses all decline with advancing age, a phenomenon called immunosenescence. For example, neutrophils and macrophages from the aged have an impaired ability to

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produce a respiratory burst and to produce nitrogen oxidants ([Table 35-1](#)). As a result, they are less able than cells from the young to kill ingested bacteria. It is more difficult to rescue neutrophils from the aged from apoptosis. Macrophage numbers decline in aged animals and they express lower levels of TLRs. Thus when stimulated with known TLR ligands, they secrete significantly reduced amounts of IL-6 and TNF- α . Aged macrophages show reduced responses to activating agents such as IFN- γ .

Dendritic cells from the aged are less effective at antigen presentation. Their reduced ability to present antigen to T cells is a result of changes in surface antigen expression and cytokine production. Their reduced ability to stimulate B cells is due to reduced immune complex binding. NK cells from the elderly are less effective at killing tumor cells. These defects in innate immunity may be more profound than defects in acquired immunity in the elderly.

Cats between 10 and 14 years of age had lower white cell, lymphocyte, and eosinophil counts than cats between 2 and 5 years of age. Absolute numbers of T cells, B cells, and NK cells were also lower in the aged animals. Serum IgA and IgM were, however, higher in the aged group. There were no differences in their complement activity or their acute-phase responses.

In Labrador dogs, absolute numbers of leukocytes, lymphocytes, monocytes, granulocytes, and CD3⁺, CD4⁺, CD8⁺, and CD21⁺ lymphocytes decreased significantly with increasing age. The relative percentages of lymphocytes and CD4⁺ cells decreased while the percentage of granulocytes and CD8⁺ cells increased. As a result, there was a significant decline in the CD4 : CD8 ratio. There was significant thymic involution, leading to a decline in the numbers of CD4⁺ T cells and in the export of cells from the thymus. In addition, these animals' peripheral lymphocyte population changed from a naïve population to a memory cell population. T cells from aged animals lose their ability to progress through the cell cycle. As a result, early events in the T cell response to antigens, such as activation of protein kinase C and the rise in intracellular calcium, are impaired. Even after expressing IL-2 receptors and being exposed to IL-2, aged T cells may not be able to respond effectively to antigens. T cells from old dogs and horses show a decline in proliferative responses to mitogens. Analysis shows that some aged T cells continue to produce normal amounts of IL-2, but many do not. Thus aged T cell populations are mixtures of fully functional and impaired cells. In old horses (older than 20 years), there is a significant decrease in the proportion of CD8⁺ T cells and a rise in the CD4 : CD8 ratio compared with young animals.

The bone marrow is relatively unaffected by old age, and an aged bone marrow can reconstitute the body as well as a young one. If aged B cells are mixed with young T cells, the response is relatively normal. If the reverse is attempted (i.e., mixing young B cells with aged T cells), the B cells respond poorly. Somatic mutation in immunoglobulin V region genes ceases in old animals so that antibody affinity tends to be lower than in young animals. Nevertheless, immunoglobulin concentrations do not decline in old age. Old dogs show little decline in antibody responses, although old horses have reduced antibody responses to influenza vaccination. Horses more than 20 years of age have reduced lymphocyte responses to mitogens, and this deficiency cannot be overcome by exposure to additional IL-2.

Despite the previous comments, young animals may show poorer resistance to some invaders than mature animals do. This seems to be especially important in sheep. Thus lambs are more susceptible than mature sheep to parasitic and infectious diseases during the first year of life. Older sheep tend to show greater resistance to internal parasites such as *Haemonchus*, *Trichostrongylus*, and *Ostertagia*. Sheep younger than 1 year of age are more susceptible than mature sheep to virus diseases such as bluetongue and contagious ecthyma. Young sheep, 4 to 8 months of age, have a lower proportion of CD4⁺ T cells in their blood than mature sheep do. Lymphocytes

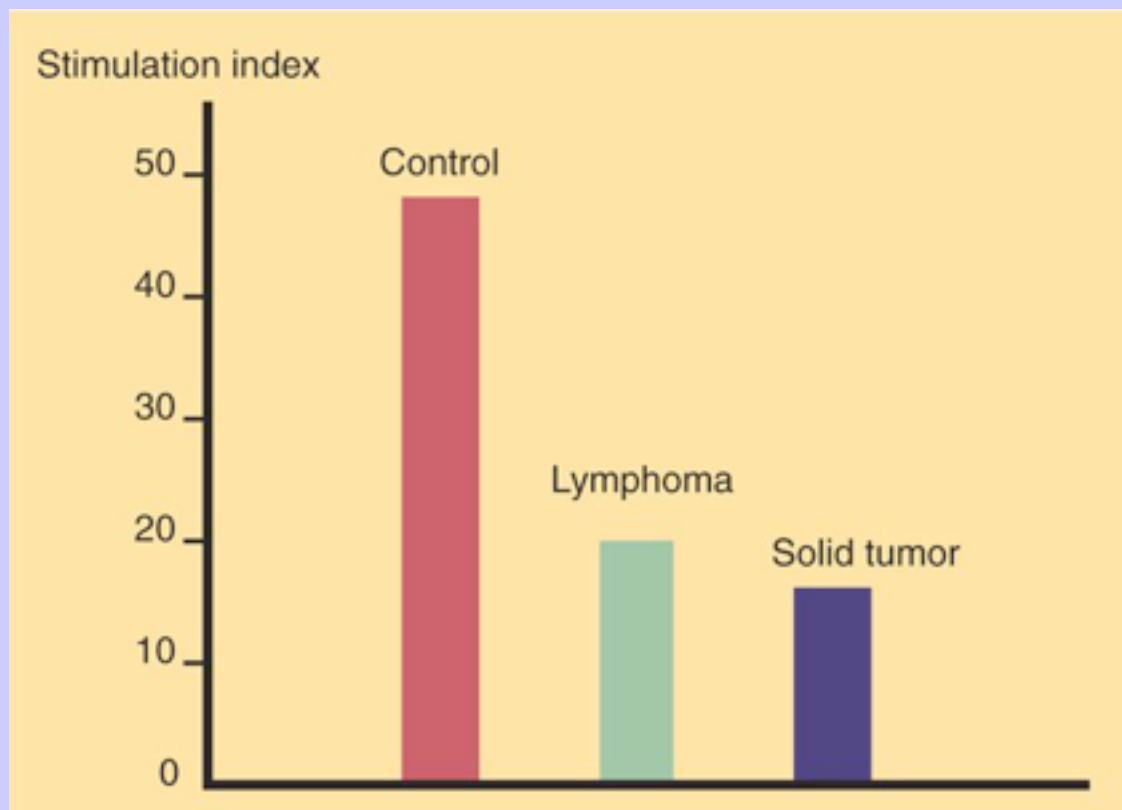
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from these young sheep produce less IFN- γ than those from adult sheep. Older sheep produce more antibodies to *Brucella abortus* lipopolysaccharide and respond more intensely to the contact sensitizer dinitrochlorobenzene. However, the two age groups do not differ in B

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FIGURE 35-7 Immunosuppression in dogs with lymphomas or solid tumors as compared to normal control dogs. The stimulation index is a measure of the response of lymphocytes to the mitogenic lectin phytohemagglutinin. (Data taken from Weiden PL, Storb R, Kolb HJ, et al: *J Natl Cancer Inst* 53:1049-1056, 1974.)



cell or WC1⁺ T cell numbers and mount comparable responses to diphtheria toxoid and tetanus. This mild immunodeficiency in lambs presumably reflects the immaturity of the immune system during the first year of life.

Feeding a diet low in calories has been shown to significantly extend the life span of dogs. One possible reason for this lengthened life span is the prevention of immunosenescence. Prolonged calorie restriction in dogs retarded age-related declines in lymphoproliferative responses, in absolute numbers of lymphocytes, and in the T, CD4, and CD8 subsets. Calorie restriction appeared to have no effect on neutrophil phagocytic activity, antibody production, or NK activity.

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35.9.7 Other Secondary Immunodeficiencies

Immunodeficiencies may result from a wide variety of insults to the body. For example, immunoglobulin synthesis is generally reduced in individuals with absolute protein loss (patients with the nephrotic syndrome, heavily parasitized or tumor-bearing individuals, and patients who have experienced severe burns or trauma). Stress may result in immunodeficiencies. For example, it is possible to provoke a combined immunodeficiency syndrome by chilling newborn puppies for 5 to 10 days. Stresses such as rapid weaning, sleep deprivation, general anesthesia, prolonged transportation, and overcrowding are all effective immunosuppressants. Physical destruction of lymphoid tissues can result in immunodeficiencies. For example, loss of lymphoid tissue leading to immunosuppression may occur in tumor-bearing animals, especially if the tumors themselves are lymphoid in origin ([Figure 35-7](#)). Adult horses with chronic diarrhea are immunosuppressed, as reflected by reduced IgA and reduced lymphocyte responses to mitogens. Some endocrine diseases such as thyrotoxicosis and diabetes mellitus may also result in immunosuppression.

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36 CHAPTER 36 Drugs and Other Agents That Affect the Immune System

36.1 KEY POINTS

- There are many drugs that can suppress immune responses. The most widely used are corticosteroids, which act on many of the cells of the immune system by preventing the activation of nuclear factor kappa-B.
- Immunosuppressive drugs used to prevent allograft rejection or for the treatment of autoimmune disease may either be nonspecific inhibitors of cell division or specifically block the activation of T cells by interfering with signal transduction.
- Drugs employed to stimulate the immune system commonly include microbial molecules that activate toll-like receptors in a very nonspecific fashion.
- Cytokine therapy has great potential, but the toxicity of these molecules presents major problems.

Many clinical situations exist in which it is desirable to stimulate or suppress the immune system, and many different drugs and techniques are available to do this. Indeed, this area of immunology, immunopharmacology, is a discipline in its own right.

36.2 SUPPRESSION OF THE IMMUNE SYSTEM

The methods available for inhibiting immune responses may be classified into two main groups. Older techniques generally involved administering treatment that, by inhibiting all cell division, reduced the re-sponse of T and B cells to antigens. This approach is crude and dangerous, since other rapidly proliferat-ing cell populations, such as intestinal epithelium and bone marrow cells, may also be severely damaged with potentially disastrous consequences. Recently, it has proved possible to selectively eliminate responding T cells by the use of specific antisera or monoclonal antibodies or by the use of highly selective immunosuppressive drugs.

36.3 NONSPECIFIC IMMUNOSUPPRESSION

36.3.1 Radiation

X-radiation can be immunosuppressive because it kills dividing cells. It affects cells by several different mechanisms. The simplest of these is through ionizing rays hitting an essential, unique molecule, such as DNA, within the cell. A loss of even one nucleotide results in a permanent mutation of a gene, with potentially lethal effects on the progeny of the affected cell. Radiation also causes ionization of water and the formation of highly reactive free oxygen and hydroxyl radicals within the cell. The hydroxyl radicals can react with dissolved oxygen to form peroxides that have toxic effects on many cell processes, especially cell division. Although X-radiation is of some use in prolonging graft survival in experimental animals, especially laboratory rodents, the amount of radiation required for effective prolongation of graft survival in the dog is so high that it is lethal to the animal.

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36.3.2 Corticosteroids

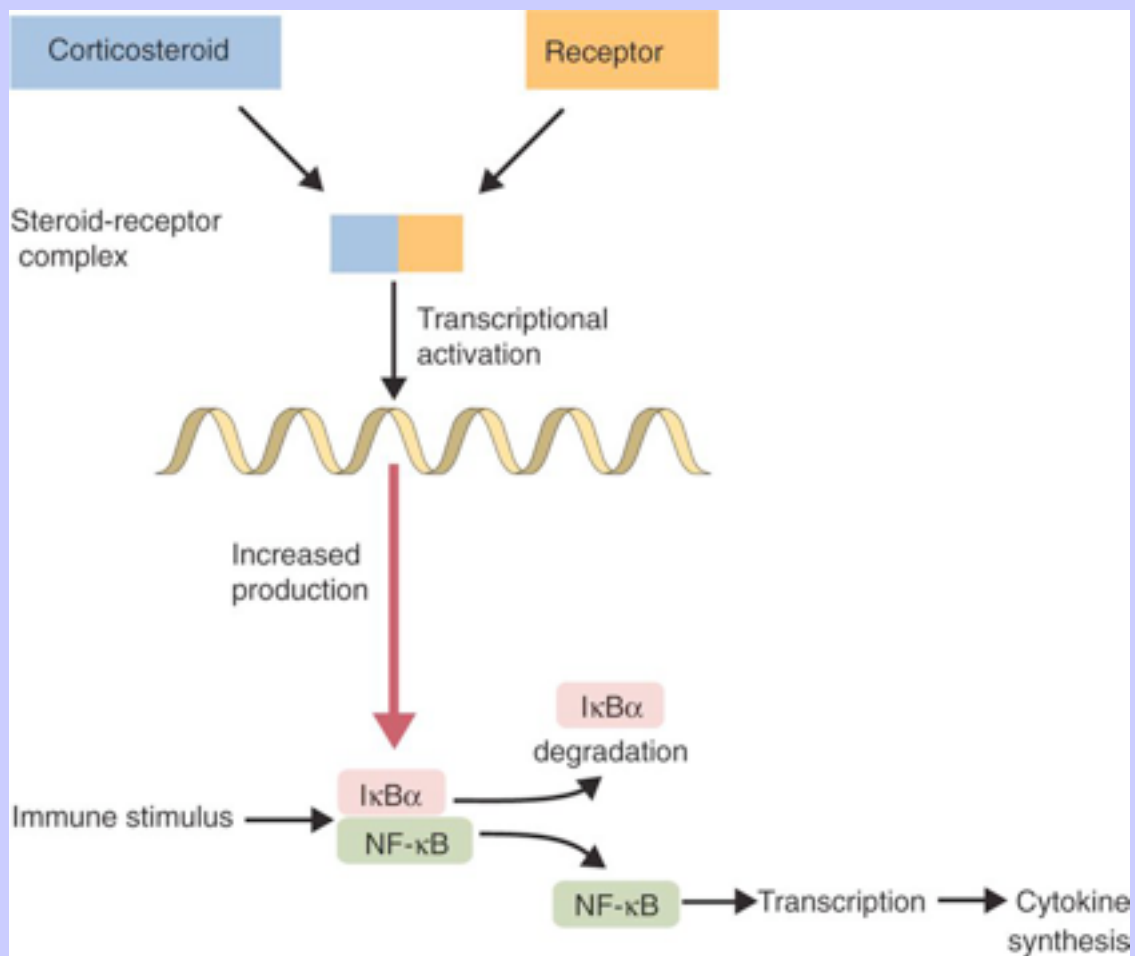
Corticosteroids are among the most commonly used immunosuppressive and antiinflammatory agents. Their potency, however, differs significantly among species. Mammals may be classified as corticosteroid-sensitive or corticosteroid-resistant on the basis of the ease by which they can be depleted of lymphocytes. Laboratory rodents and humans are much more sensitive to the immunosuppressive effects of corticosteroids than the major domestic mammals, and care should therefore be taken not to extrapolate laboratory animal results directly to other species.

The effects of glucocorticosteroids on cell function have a common pathway ([Figure 36-1](#)). Corticosteroids are absorbed directly into cells, where they bind to cytoplasmic receptors. The corticosteroid-receptor complexes are then transported to the nucleus, where they stimulate the synthesis of I κ B α , the inhibitor of nuclear factor kappa-B (NF- κ B). In a resting cell, NF- κ B is inactive since its nuclear binding site is masked by I κ B α . When a lymphocyte is stimulated, the two molecules dissociate, the I κ B α is degraded by ubiquitination and proteasomes, and the released NF- κ B moves to the nucleus and acts as a transcription factor, activating many genes involved in inflammation and immunity. Corticosteroids, however, stimulate the production of excess I κ B α . This excess is not degraded but rebinds to NF- κ B and continues to block all NF- κ B-mediated processes, including cytokine synthesis and T cell responses. As a result, corticosteroids suppress both immunological and inflammatory processes.

Corticosteroids influence immunity in four areas ([Box 36-1](#)): They have effects on leukocyte circulation; they influence the effector mechanisms of lymphocytes; they modulate the activities of inflammatory mediators; and they modify protein, carbohydrate, and fat metabolism.

The effects of corticosteroids on leukocyte circulation vary among species. In horses and cattle, the

FIGURE 36-1 A schematic diagram showing the mode of action of corticosteroids. Normally, signal transduction and cytokine synthesis occur when the transcription factor nuclear factor kappa-B (NF- κ B) dissociates from its inhibitor I κ B α . The released I κ B α is rapidly degraded. Glucocorticosteroids stimulate the synthesis of excessive amounts of I κ B α , which binds to NF- κ B and so continues to prevent its activation.



number of circulating eosinophils, basophils, and lymphocytes declines within a few hours of corticosteroid administration as a result of sequestration in the bone marrow. The numbers of neutrophils, on the other hand, increase as a result of decreased adherence to vascular endothelium and reduced emigration into inflamed tissues. Neutrophil, monocyte, and eosinophil chemotaxis is suppressed by corticosteroids, but neutrophil random migration is enhanced. Corticosteroids suppress the cytotoxic and phagocytic abilities of neutrophils in some species, but in others, such as the horse and goat, they have no effect on phagocytosis. Macrophage production of prostaglandins and cytokines such as interleukin-1 as well as antigen processing is reduced in some species.

36.3.2.1 Box 36-1 The Effects of Corticosteroids on the Immune System

36.3.2.1.1 Neutrophilia

- Depressed chemotaxis
- Depressed margination
- Depressed phagocytosis
- Depressed antibody-dependent cell-mediated cytotoxicity
- Depressed bactericidal activity

36.3.2.1.2 Macrophages

- Depressed chemotaxis
- Depressed phagocytosis
- Depressed bactericidal activity
- Depressed interleukin-1 production
- Depressed antigen processing

36.3.2.1.3 Lymphocytes

- Depressed proliferation
- Depressed T cell responses
- Impaired T cell-mediated cytotoxicity
- Depressed interleukin-2 production
- Depressed lymphokine production

36.3.2.1.4 Immunoglobulins

- Minimal decrease

36.3.2.1.5 Complement

- No effect

Glucocorticosteroids cause apoptosis of thymocytes, especially those with the double-positive phenotype (CD4⁺, CD8⁺). They also suppress the ability of T cells to produce cytokines. The most important exception to this is interleukin-2 (IL-2), which is not regulated by NF-κB. Lymphocyte proliferation in the mixed lymphocyte

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reaction is suppressed, suggesting that there is interference with the recognition of major histocompatibility complex (MHC) class II molecules. Corticosteroids also block production of lymphotoxin and monocyte chemotactic molecules. Natural killer and some antibody-dependent cellular cytotoxicity reactions may be refractory to corticosteroid treatment, and in cattle corticosteroids may increase serum interferon levels. The effects of corticosteroid therapy on antibody responses are variable and depend on the timing and the dose given. In general, B cells tend to be corticosteroid-resistant, and enormous doses are usually required to depress antibody synthesis. It is interesting to note, however, that in horses, moderate doses of dexamethasone suppresses immunoglobulin G1 (IgG1) and IgG4 responses while having no apparent effect on IgG3 responses. Corticosteroids also upregulate the expression of CD121b. This is a decoy receptor that can bind active IL-1 but will not transduce a signal, effectively suppressing the activity of IL-1.

The synthetic glucocorticoids are able to suppress acute inflammation. They inhibit the increase in vascular permeability and vasodilatation. As a result they prevent edema formation and fibrin deposition. At the same time, corticosteroids block the emigration of leukocytes from capillaries. They inhibit the release of lysosomal enzymes and impair antigen processing by macrophages. Corticosteroids can also inhibit the effects of phospholipases and so prevent the production of leukotrienes and prostaglandins. These effects of corticosteroids may mask signs of tissue damage. In the later stages of inflammation, they inhibit capillary and fibroblast proliferation (perhaps by blocking IL-1 production) and enhance collagen breakdown. As a result, corticosteroids delay wound and fracture healing.

When corticosteroid therapy is initiated, prednisolone is usually the agent selected for small animal treatment, and betamethasone and dexamethasone are commonly employed in large animal practice. Cats may require significantly higher doses than dogs to achieve a significant clinical response. Once a response has been induced, the dose of corticosteroids can be gradually reduced by lengthening the dose interval and then decreasing the amount given. This treatment is not without risks since it has the potential to suppress the pituitary-adrenal axis and induce Cushing's syndrome. By suppressing inflammation and phagocytosis, corticosteroids may render animals highly susceptible to infection.

36.3.3 Cytotoxic Drugs

The major immunosuppressive cytotoxic drugs are designed to inhibit cell division by blocking nucleic acid synthesis and activity. The major cytotoxic drugs currently in use are alkylating agents, folic acid antagonists, and DNA synthesis inhibitors.

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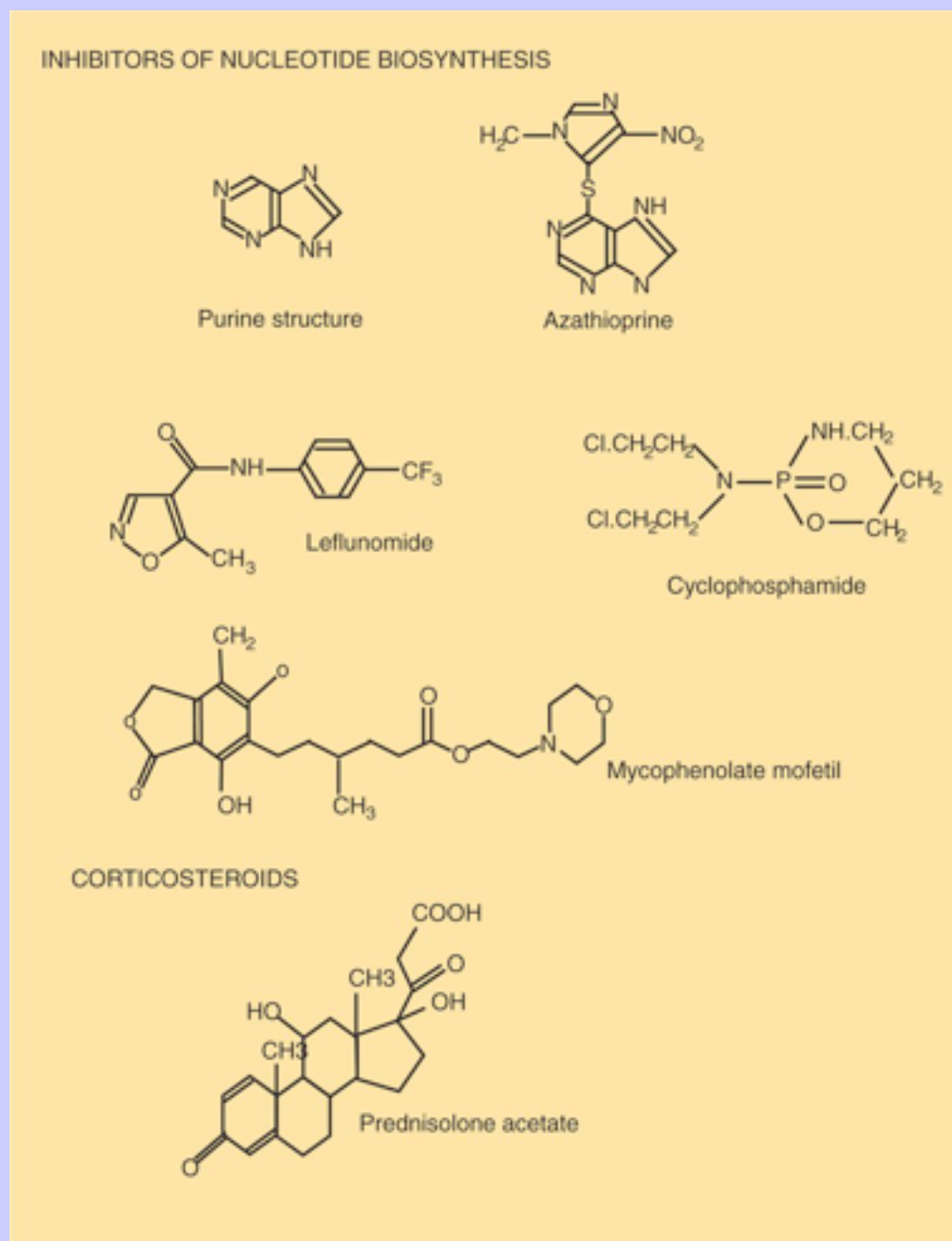
36.3.3.1 Alkylating Agents

The alkylating agents cross-link DNA helices, preventing their separation and thus inhibiting template formation. The most important of these is cyclophosphamide ([Figure 36-2](#)). Cyclophosphamide is toxic for resting and dividing cells, especially for dividing immunocompetent cells. It impairs both B and T cell responses, especially the primary immune response. It blocks mitogen and antigen-induced cell division and the production of cytokines such as interferon- γ (IFN- γ). It prevents the B cell from renewing its antigen receptors. Early in therapy, cyclophosphamide tends to destroy more B cells than T cells. In long-term therapy it affects both cell populations. It also suppresses macrophage function and therefore has an antiinflammatory effect. Cyclophosphamide may be administered parenterally or orally and is inactive until biotransformed in the liver. It has a half-life of about 6 hours and is largely excreted through the kidney. It is of interest to note that corticosteroids enhance the metabolism of cyclophosphamide and so reduce its potency. The main toxic effect of cyclophosphamide is bone marrow suppression, leading to leukopenia with a predisposition to

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infection. Other effects may include thrombocytopenia, anemia, and bladder damage. Cyclophosphamide is of benefit in the treatment of lymphoid neoplasia and in the treatment of immune-mediated skin diseases.

FIGURE 36-2 The structure of some commonly employed immunosuppressive drugs and the normal compounds with which they compete. Cyclophosphamide acts by cross-linking DNA chains.



36.3.3.2 Folic Acid Antagonists

Methotrexate is a folic acid antagonist that binds to dihydrofolate reductase and so blocks the synthesis of tetrahydrofolate, leading to failure to synthesize thymidine and purine nucleotides. As a result, it can suppress antibody formation. Its side effects are similar to those seen with cyclophosphamide.

36.3.3.3 DNA Synthesis Inhibitors

Azathioprine is a nucleoside analog that suppresses activated lymphocytes. It is metabolized in the liver to thio-inosine-monophosphate, which inhibits adenylic and guanylic acid production. T and B cells are especially susceptible to this effect. It can suppress both primary and secondary antibody responses if given after antigen exposure. Azathioprine has a significant antiinflammatory action as a result of its ability to inhibit the production of macrophages. It has no effect on the production of cytokines by lymphocytes and affects both T and B responses equally. Its major toxic effect is bone marrow depression, which tends to affect leukocytes rather than platelets or red cells. Azathioprine is useful in the control of allograft rejection but is ineffective in preventing xenograft rejection. It is favored by many clinicians for the treatment of immune-mediated skin diseases because of its combination of antiinflammatory and immunosuppressive activity. It is commonly used in association with corticosteroid therapy. If azathioprine is used in dogs, marrow function should be carefully monitored and the dose reduced if adverse effects occur.

36.4 SELECTIVE IMMUNOSUPPRESSION

36.4.1 Calcineurin Inhibitors

Perhaps the single most important step in the development of routine, successful organ allografting has been the development of potent but selective immunosuppressive agents. Of these, cyclosporine has been by far the most successful. Cyclosporine is an immunosuppressive polypeptide derived from certain fungi. These fungi yield several natural forms of cyclosporin, of which the most important is cyclosporin A, a peptide consisting of 11 amino acids arranged in a circle ([Figure 36-3](#)). Because of this structure, cyclosporine has two distinct surfaces, or faces, which allows it to bind two proteins simultaneously. When it enters the cytoplasm, one face binds to an intracellular receptor called cytophilin, while the other face binds and blocks the intracellular transmitter calcineurin, a serine/threonine phosphatase. As a result, cyclosporine inhibits signal transduction and blocks production of IL-2 and IFN- γ by T cells. The net effect of cyclosporine treatment is therefore the blocking of Th1 responses.

Because cyclosporine inhibits the production of IFN- γ by activated T cells, it will block IFN- γ induction of MHC class I expression in grafts. Since corticosteroids have a similar effect, the combination of corticosteroids and cyclosporine is especially potent and can enhance survival of allografts while at the same time leaving other immune functions intact. It therefore has a significant advantage over other older immunosuppressants. The use of cyclosporine has made tissue transplantation a routinely successful and safe procedure. In cats that have received renal allografts from unrelated blood-group-compatible donors and that were treated with cyclosporine and prednisolone, mean survival is greater than 12 months.

Cyclosporine also inhibits several hypersensitivity reactions. Thus it is as effective as corticosteroids in treating canine atopic dermatitis. It is useful in a variety of immunologically mediated dermatological diseases and appears to have a wide safety margin in dogs.

Tacrolimus is a macrolide antibiotic that acts as a calcineurin blocking agent in a manner similar to cyclosporine (see [Figure 36-3](#)). It inhibits the production of several key cytokines, including IL-2, IL-3, IL-4, IL-5, IFN- γ , and tumor necrosis factor- α (TNF- α). Tacrolimus is much more potent than cyclosporine in inhibiting T and B cell responses. It is also superior to cyclosporine in preventing or reversing allograft and xenograft rejection in humans and can prevent graft vascular disease (see [Chapter 29](#)). Unfortunately, it causes severe intestinal toxicity in dogs, resulting in ulceration, vasculitis, anorexia, and vomiting. Topical tacrolimus has been used successfully to treat discoid lupus erythematosus and pemphigus erythematosus in dogs.

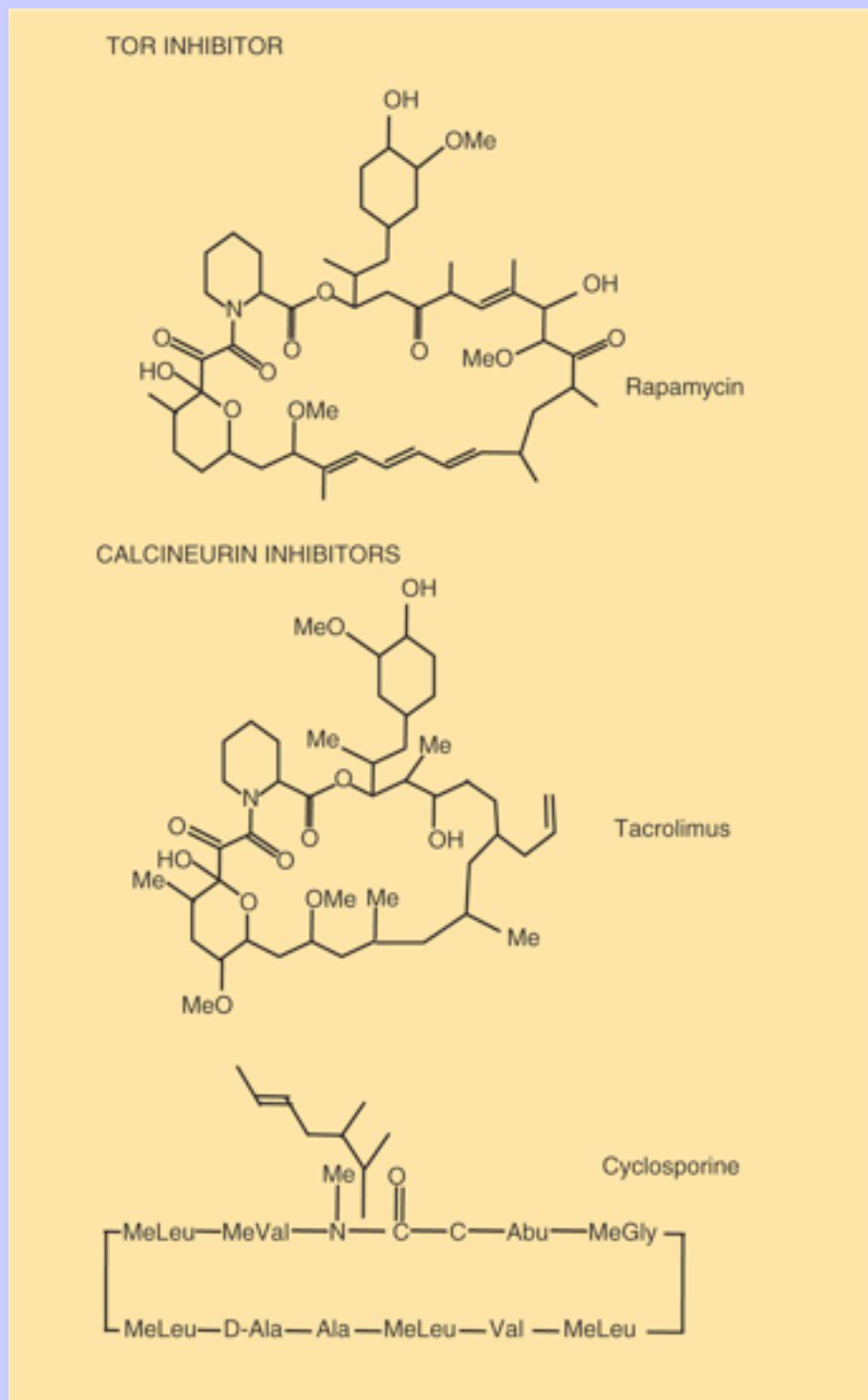
36.4.2

Inosine Monophosphate Dehydrogenase Inhibitors

Mycophenolate mofetil significantly prolongs canine allograft survival but is severely toxic to the canine gastrointestinal tract. It acts selectively on activated lymphocytes since it preferentially inhibits an isoform of the purine pathway enzyme inositol monophosphate dehydrogenase found in activated but not resting lymphocytes. This leads to reduced production of guanosine monophosphate and blockage of the production of DNA. When given with cyclosporine, mycophenolate mofetil completely prevents renal allograft rejection between unrelated mongrel dogs. It is very effective in controlling canine autoimmune diseases such as immune-mediated thrombocytopenia, autoimmune hemolytic anemia, nonsuppurative meningoencephalitis, polymyositis, and pemphigus

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FIGURE 36-3 The structure of the calcineurin inhibitors, rapamycin, tacrolimus, and cyclosporine. Abu, Aminobutyric acid.



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foliaceus, as well as systemic histiocytosis (see [Chapter 8](#)).

36.4.3 Target of Rapamycin Inhibitors

The macrolide antibiotic rapamycin (sirolimus) and a related molecule everolimus inhibit a serine protein kinase known as mammalian target of rapamycin (TOR). TOR plays a critical role in integrating the signals that determine whether T cell antigen recognition will lead to activation or anergy. Rapamycin inhibits B and T cell proliferation by blocking stimulatory signals from IL-2, IL-4, and IL-6. Sirolimus acts synergistically with calcineurin inhibitors and is much superior to cyclosporine in preventing or reversing allograft and xenograft rejection in humans. Because it blocks endothelial cell and fibroblast proliferation, it can prevent graft vascular disease (see [Chapter 30](#)) although it also inhibits wound healing. Unfortunately, it also induces severe intestinal toxicity in dogs, causing ulceration, vasculitis, anorexia, and vomiting.

36.4.4 Other Immunosuppressive Drugs

Leflunamide is an antiinflammatory agent widely employed in the treatment of inflammatory disorders rather than in preventing allograft rejection. Other newly developed immunosuppressive drugs that may be useful in veterinary medicine include 15-deoxyspergualin, a spermidine polyamine that binds to heat-shock protein 70 and interferes with antigen processing, and brequinar sodium, which is an inhibitor of pyrimidine synthesis.

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36.4.5 Depletion of Lymphocytes

Because of the many adverse side effects of the nonspecific cytotoxic drugs (not the least important of which is an increased predisposition to infection), a considerable effort has been made to find more specific alternative immunosuppressive procedures. One relatively simple technique that largely depletes T cells is to administer an antiserum specific for T lymphocytes. Antilymphocytic serum (ALS) suppresses the cell-mediated immune response and leaves the humoral immune response relatively intact. In practice, ALS has proved to be of variable efficiency and causes severe side effects. ALS-treated mice have been shown to accept rat xenografts, whereas clinical use of ALS in humans has not been universally accepted as useful. A much more specific antiserum with precise targeting is monoclonal anti-CD3. Anti-CD3 is directed only against T cells and appears to be very effective in reversing graft rejection in humans. An even more specific monoclonal antibody is anti-CD25. This binds to the α chain of the IL-2 receptor and thus prevents lymphocyte activation. Anti-CD25 helps prevent renal allograft rejection and, since it does not cause T cell depletion, has fewer side effects and leads to fewer opportunistic infections than polyclonal antilymphocyte globulin.

Monoclonal antibodies against canine CD4 and CD8 have been used to control rejection of canine renal allografts. They are very effective, even with highly mismatched mongrel dogs. Both anti-CD4 and anti-CD8 must be used together, and their immunosuppressive effect lasts for about 10 days. (The dogs develop neutralizing antibodies against these monoclonal antibodies.) These are especially effective in combination with cyclosporine, since this reduces IL-2 production.

In some diseases, especially those due to excessive immune function, it may be beneficial to neutralize excessive cytokine activity using monoclonal antibodies. Thus it is possible to use highly neutralizing antibodies against a cytokine or against its receptor. The most successful anticytokine antibodies employed in humans are those directed against TNF- α (infliximab). These are used to suppress inflammation in rheumatoid arthritis and Crohn's disease.

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Although immunoglobulin replacement is appropriate for animals with antibody deficiencies, immunoglobulin therapy also appears to be immunosuppressive in some autoimmune diseases. Thus intravenous human immunoglobulin has been used to treat successfully severe pemphigus foliaceus, Gullain-Barré syndrome, immune-mediated thrombocytopenia, and immune-mediated hemolytic anemia in dogs. Its mechanism of action is unknown, but it may function by blockading FcRn and thus accelerating autoantibody degradation (see [Chapter 17](#)).

36.5 STIMULATION OF THE IMMUNE SYSTEM

There are many situations in veterinary medicine in which it is desirable to enhance innate or acquired immunity: for example, the enhancement of resistance to infection and the treatment of immunosuppressive diseases. Immunostimulants vary according to their origin, their mode of action, and how they are used. In contrast to adjuvants, immunostimulants need not be administered together with an antigen to enhance an immune response.

36.5.1 Bacteria and Bacterial Products

A wide variety of bacteria have been employed as immunostimulants. These most probably bind and stimulate one or more toll-like receptors (TLRs). As a result, they activate macrophages and dendritic cells and stimulate cytokine synthesis. Their immunostimulating effects are probably due to the release of a mixture of cytokines. The most potent of these cytokine synthesis enhancers is bacillus Calmette-Guérin (BCG), the live, attenuated vaccine strain of *Mycobacterium bovis*. BCG generally enhances B and T cell-mediated responses, phagocytosis, graft rejection, and resistance to infection. Unfortunately, whole BCG induces tuberculin hypersensitivity in treated animals and is therefore unacceptable for use in farm animals. In order to prevent sensitization, purified cell wall fractions of BCG have been employed. These have been used to treat equine sarcoids and ocular squamous cell carcinoma. They are also of benefit in the treatment of upper respiratory tract infections in horses. Fractionation of BCG has resulted in the isolation of several active constituents. One of these is trehalose dimycolate. It promotes nonspecific immunity against several bacterial infections and may provoke regression of some experimental tumors. Muramyl dipeptide (MDP), a simple glycopeptide also purified from *Mycobacteria*, enhances antibody production, stimulates polyclonal activation of lymphocytes, and activates macrophages. Because MDP is rapidly excreted in the urine, its biological activity is greatly enhanced by incorporation into liposomes. Polymerization and conjugation with glycopeptides or synthetic antigens can also enhance the immunostimulating effects of MDP. MDP has been shown to be of benefit in prolonging survival time and decreasing metastases in dogs with osteosarcoma.

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Killed anaerobic corynebacteria, such as *Propionibacterium acnes*, promote antibody formation. These bacteria are phagocytosed by macrophages and presumably stimulate cytokine synthesis through TLRs. *P. acnes* has a complex activity since it stimulates macrophages and the antibody response to thymus-dependent antigens, but it has a variable effect on the response to thymus-independent antigens. (It may selectively promote a Th2 response.) These organisms have a general immunostimulatory action, leading to enhanced antibacterial and antitumor activity. Killed *P. acnes* has been of benefit in the treatment of staphylococcal pyoderma, malignant oral melanoma in dogs, feline leukemia in cats, and respiratory disease in horses.

Staphylococcal cell walls (especially staphylococcal phage lysate), some streptococcal components, and components of *Bordetella pertussis*, *Brucella abortus*, *Bacillus subtilis*, and *Klebsiella pneumoniae* all have immunostimulative activity.

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Unmethylated cytosine-guanosine nucleotides can bind to the dendritic cell/macrophage receptor TLR9, activate antigen-presenting cells, and trigger a potent Th1 cytokine response. When administered with antigens, these nucleotides act as potent adjuvants. When administered alone, they can act as immunostimulants and greatly enhance innate immunity.

36.5.2 Complex Carbohydrates

Certain complex carbohydrates derived from yeasts—namely, zymosan, glucans, aminated polyglucose, and lentinans—can also activate macrophages. These may function as adjuvants and potentiate resistance to infectious agents. Acemannan, a complex carbohydrate derived from the Aloe vera plant, is a cytokine synthesis enhancer with antitumor and antiviral activities. It has been used to treat feline leukemia and fibrosarcomas in cats and dogs. Fish such as trout, salmon, and catfish appear to respond well to these immunostimulants when incorporated into the diet. As a result, immunostimulation by carbohydrates, especially glucans, is routine in many aquaculture operations.

36.5.3 Immunoenhancing Drugs

Levamisole, a broad-spectrum anthelmintic, functions in a manner similar to the thymic hormone thymopoietin (see [Chapter 10](#)); that is, it stimulates T cell differentiation and T cell response to antigens. Levamisole enhances bovine lymphocyte blastogenesis at sub-optimal mitogen concentrations, enhances interferon production, and increases FcR activity in bovine macrophages. It probably also enhances cell-mediated cytotoxicity, lymphokine production, and suppressor cell function. Levamisole stimulates the phagocytic activities of macrophages and neutrophils. Its effects are greatest in animals with depressed T cell function; it has little or no effect on the immune system of healthy animals. Levamisole may therefore be of assistance in the treatment of chronic infections and neoplastic diseases but may exacerbate disease caused by excessive T cell function.

36.5.4 Vitamins

Vitamin E and selenium affect immune responses and disease resistance in poultry, pigs, and laboratory animals. A deficiency of vitamin E ([dl]- α -tocopheryl-acetate) results in immunosuppression and reduced resistance to disease. On the other hand, supplementation of diets with vitamin E can enhance certain immune responses and lead to increased resistance to disease. Lymphocyte responses to pokeweed mitogen are higher in pigs with high vitamin E levels. Vitamin E supplementation given to cows for several weeks before calving prevents the decline in neutrophil function (superoxide production) and macrophage function (IL-1 production and MHC class II expression) that normally occurs in the immediate postparturient period. Vitamin E promotes B cell proliferation; the effect is most marked in the primary immune response. It can act as an adjuvant when administered with *Brucella ovis* vaccine, *clostridial toxoid*, and *Escherichia coli* J5 vaccine. In some cases this increased antibody production may lead to increased disease resistance. The mode of action of vitamin E in enhancing immunity is unclear, but supplemental vitamin E may act as a significant stimulus of immunity in some animals.

36.5.5 Cytokines

Since purified cytokines produced by recombinant DNA techniques are now available, many investigators have studied whether they can be used to treat disease. By administering additional cytokines, one assumes that the amount of these molecules in the normal animal is rate-limiting and that administering additional material in

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pure form will somehow promote disease resistance or healing. This also assumes that, by administering a single new cytokine, one will not trigger mechanisms that will regulate its activity and perhaps neutralize its effects. None of these assumptions may be valid. The major cytokines (IL-1, IL-2, IL-12, colony-stimulating factors, and the interferons) have all been tested on animals in vivo. Unfortunately, the administration of purified cytokines has usually had minimal effects on disease processes and has been accompanied by significant adverse effects.

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36.5.5.1

Interferons

It was predicted for many years that the interferons would prove to be effective antiviral agents if they were made available in large quantities for animal treatment. Theoretically, administration of interferons should inhibit virus replication as well as stimulate some cellular functions such as neutrophil activity, thereby promoting disease resistance. This has proved to be an oversimplification. High doses of interferons are very toxic and cause severe fever, malaise, and appetite loss. They inhibit hematopoiesis and so cause thrombocytopenia and granulocytopenia. They can also cause liver, kidney, and neural toxicity. In addition, interferons seem to be relatively poor antiviral agents.

Recombinant human IFN- α (rHuIFN- α) is the treatment of choice for hepatitis B and C in humans. It has been administered to calves to treat rhinopneumonitis caused by bovine herpesvirus 1 (BHV-1). Although treated calves were less severely affected than controls, toxic effects such as fever and depression were seen. Multiple doses were required, and some calves developed antibodies against the interferon. rHuIFN- α has also been used to treat rotavirus-induced diarrhea in calves. It caused some clinical improvement but had no effect on virus shedding. Recombinant bovine interferon (rBoIFN- α or rBoIFN- γ) has also been administered to calves. rBoIFN- α significantly reduced disease symptoms in calves experimentally inoculated with BHV-1 and *Mannheimia haemolytica*. It was ineffective when given orally for the treatment of experimental transmissible gastroenteritis in piglets. Prophylactic treatment of healthy calves with rBoIFN- α 1 significantly reduced the incidence of respiratory disease in these animals. rBoIFN- γ increased the secondary antibody response to vesicular stomatitis virus. It also enhanced neutrophil-mediated killing of *B. abortus*, but it had a mixed effect on the ability of calves to survive challenge with virulent *Salmonella* serotype *typhimurium*, since some treatments reduced calf survival. Recombinant porcine IFN- γ decreased the mortality of pigs challenged with *Actinobacillus pleuropneumoniae*. Dairy cattle that received intramammary rBoIFN- γ 24 hours before challenge with *E. coli* had considerably less mastitis than untreated controls. There was no mortality in the treated group, but the control group had 42% mortality within 3 days. Porcine IFN- α (PoIFN- α) is a powerful adjuvant for a recombinant protein vaccine against foot-and-mouth disease virus in swine.

It is possible that interferons may have a more consistent positive therapeutic effect if, instead of being used on healthy animals, they are used on immunosuppressed, stressed animals. Glucocorticosteroids decrease the ability of animals to produce interferon, and rBoIFN- γ does reduce the severity of *Histophilus somni*-induced pneumonia in dexamethasone-treated calves.

Human and bovine IFN- α have been used for the treatment of feline leukemia. The most impressive results were obtained with low-dose oral IFN- α in experimentally infected cats, in which significant protection was observed. IFN- γ has been used to treat canine parvoviral enteritis. Recombinant feline IFN- ω has also been tested for its ability to treat disease due to feline leukemia virus or feline immunodeficiency virus infections. It appears to be of benefit in significantly reducing the clinical severity of disease and increasing cat survival.

In conclusion, the use of high doses of interferons for the treatment of infectious diseases of cattle and swine has produced some positive responses. These are, however, not impressive, and the interferon induces major

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toxic side effects such as fever, inappetence, and malaise. Low doses of interferon administered orally may produce more consistent positive results without these adverse side effects.

36.5.5.2

Interleukins

Recombinant IL-2 was administered to animals at the same time that they were vaccinated against a variety of organisms. In general this resulted in an increased level of protection. For example, rHuIL-2 administered to pigs at the same time that they received an *A. pleuropneumoniae* bacterin induced a considerably greater protection on challenge. A similar result was obtained using pigs immunized with a pseudorabies subunit vaccine. Calves that were vaccinated against BHV-1 and injected with rBoIL-2 showed enhanced responses to the virus and showed less severe signs of infection after challenge. Unfortunately, IL-2 is very toxic. It causes severe side effects, including malaise, a capillary leak syndrome, diarrhea, and fever (see [Chapter 30](#)). Intramammary infusions of rBoIL-2 do induce local macrophage and neutrophil infiltration and increase mastitis cure rates. It is also interesting to note that relatively low doses of rHuIL-2, when injected locally into papillomas or carcinomas of the vulva in cattle, induced a positive response in over 80% of cases and some complete regressions were observed.

36.5.5.3

Other Cytokines

In addition to the trials described above, studies have been conducted using IL-1 and granulocyte-macrophage colony-stimulating factor (GM-CSF). rBoIL-1 β treatment of calves simultaneously vaccinated against BHV-1 resulted in no change in disease severity, although it is an effective adjuvant in BHV-1 immunization. OvIL-1 β has been used to enhance the response of sheep to purified, adjuvanted blowfly antigens. Although this treatment did not increase antibody levels, the animals showed enhanced delayed hypersensitivity and protection. It also appeared to have a protective effect against *Streptococcus suis* challenge in pigs. rBoGM-CSF increased neutrophil functions in corticosteroid-treated calves, enhancing their ability to phagocytose *Staphylococcus aureus* and suggesting that it might be useful in disease treatment. Encouraging results have also been obtained by the administration of IL-12, which promotes Th1 cell function. Administration of IL-12 to pigs receiving an inactivated pseudorabies vaccine effectively enhances their cell-mediated responses. BoIL-12 has helped to promote a Th1 response to *Salmonella* serotype *typhimurium* in calves. Despite this, the clinical trials of purified cytokines have generally produced disappointing results.

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36.6

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37 CHAPTER 37 The Evolution of the Immune System

37.1 KEY POINTS

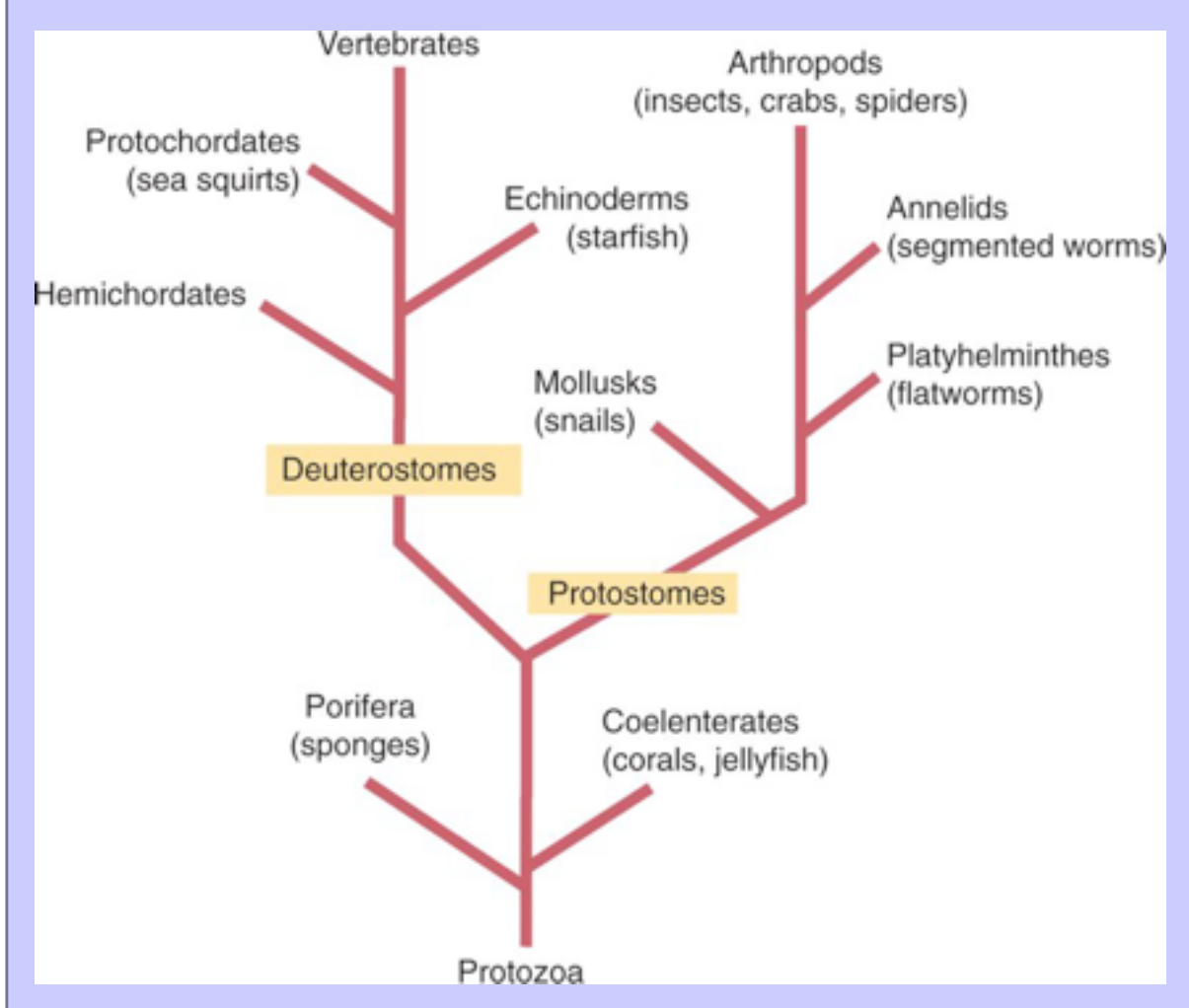
- Invertebrates rely exclusively on the use of innate immune mechanisms to protect themselves against infectious agents.
- The jawless fish also rely exclusively on innate immunity, although they do possess a diverse antigen-binding receptor system.
- Cartilaginous and bony fish are the first vertebrates to have evolved an acquired immune system. It is suggested that this occurred relatively suddenly during the evolutionary process with the incorporation into the fish genome of a microbial transposon containing recombinase genes.
- Jawed fish and all the more evolved vertebrates possess both antibody and cell-mediated immune systems, although the details differ between species.
- The major chicken immunoglobulin is called immunoglobulin Y (IgY) because it is structurally different from mammalian IgG.

All animals, regardless of their complexity or evolutionary history, must be able to defend themselves against invading organisms that might cause disease or death. Both invertebrates and vertebrates possess innate immune defenses triggered by “danger signals” such as tissue damage or microbial invasion. The acquired immune system, however, evolved only after the emergence of the jawless fishes or cyclostomes. Thus, acquired immune mechanisms such as antibody production or antigen-responsive lymphocytes are found only in the advanced vertebrates.

37.2 IMMUNITY IN INVERTEBRATES

Invertebrates are classified based on the presence of a body cavity or coelom ([Figure 37-1](#)). The acoelomates include the sponges and coelenterates (jellyfish and sea anemones). The coelomates evolved further into two major lines. One line includes the annelids,

FIGURE 37-1 A phylogenetic tree showing the major divisions of the invertebrates.



mollusks, and arthropods, collectively called the protostomes. The other line, including the echinoderms, protochordates, and chordates, is called the deuterostomes. It is from deuterostome-like ancestors that the vertebrates evolved. Invertebrates rely exclusively on physical barriers and innate immune defenses to exclude microbial invaders.

37.2.1 Physical Barriers

Physical barriers are most obvious in the arthropods. Tough, chitinous exoskeletons can protect arthropods against all types of attackers. The horseshoe crab (*Limulus polyphemus*) not only has a hard exoskeleton but also can protect itself against bacteria in polluted water by secreting a specialized glycoprotein through pores in the carapace. On contact with endotoxins, this glycoprotein coagulates, sealing the pores and immobilizing any invading bacteria. Likewise, if bacteria enter horseshoe crab hemolymph, clotting factors are activated by lipopolysaccharides and result in local clot formation that traps invaders. Other invertebrates such as the

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coelenterates, annelids, mollusks, and echinoderms secrete masses of sticky mucus when attacked, thus immobilizing potential invaders. This mucus may contain defensins and other anti-microbial peptides.

37.2.2 Innate Immunity

Invertebrates use three major innate defense mechanisms: phagocytosis by blood or body cavity cells; protease cascades that lead to fluid clotting, melanin formation, and opsonization; and the production of a wide variety of antimicrobial peptides. Because of their dependence on innate immunity, invertebrates have evolved multiple complex pattern recognition receptors. Thus in the sea urchin (*Strongylocentrotus purpuratus*) there are 222 different toll-like receptor (TLR) genes and more than 200 NOD-like genes. TLRs have been identified in even the least evolved invertebrates such as the sponges.

37.2.2.1 Phagocytosis

In 1884 Eli Mechnikoff discovered phagocytosis when examining starfish larvae. He showed that mobile cells attacked rose thorns introduced into the coelom of these larvae. Since then, phagocytosis has been shown to be a universal defense mechanism within the animal kingdom. Several different types of phagocytic cells are recognized in coelomate invertebrates. They occur in blood (hemocytes) and in the body cavity (coelomocytes). These cells behave like mammalian phagocytes and undertake chemotaxis, adherence, ingestion, and digestion. They contain proteases and in some invertebrates, such as mollusks, they produce potent oxidants. Some phagocytes can aggregate and plug wounds to prevent bleeding. In some cases, where phagocytic cells cannot control them, invaders may be walled off in cellular nodules somewhat similar to vertebrate granulomas.

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Invertebrates can produce cytokine-like molecules. One of these, an interleukin-1 (IL-1)-like molecule, may activate phagocytic cells and stimulate phagocytosis. Lipopolysaccharide stimulation of mollusk hemocytes may stimulate the release of tumor necrosis factor (TNF)-like, IL-6-like, or IL-1-like proteins. Cell surface adhesive proteins such as integrins are found in arthropods such as *Drosophila* or freshwater crayfish. These may promote hemocyte degranulation and activation of the prophenoloxidase system.

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37.2.2.2 The Prophenoloxidase-Activating System

The prophenoloxidase (proPO)-activating system, found in arthropod hemolymph, consists of multiple enzymes that, when activated, generate a cascade of proteases leading to the production of the inert polymeric pigment melanin ([Figure 37-2](#)). The system is activated by the interaction of bacterial and fungal lipopolysaccharides, peptidoglycans, and glucans with hemocytes. Activation also occurs through cuticular and hemolymph proteases. The proPO system generates phenoloxidase, a sticky enzyme that binds to foreign surfaces. This enzyme acts on tyrosine and dopamine to generate melanin and deposit it around inflammatory sites. Melanin polymer is deposited in the tissues surrounding invaders to form an impermeable barrier that blocks their nutrient uptake. Oxidizing agents and other antimicrobial molecules are generated during melanin synthesis.

37.2.2.3 Antimicrobial Peptides

When insects are injected with bacteria, their PAMPs are recognized by toll and other receptors. In contrast to mammals, where TLRs directly recognize pathogens, *Drosophila* toll is activated by a protein ligand (called spätzle) that is generated after pathogen recognition. As a result of activation of these pathways, arthropod

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cells produce diverse antimicrobial peptides. These peptides are mainly produced in the fat body (the functional equivalent of the mammalian liver), although some may be produced locally on body surfaces. The peptides appear about 2 hours after exposure to the bacteria and reach peak levels at 24 hours. In some insects, the activity is short-lived and disappears in a few days; in others, it may last for several months. About 400 different antimicrobial peptides, including the defensins, have been identified in invertebrates. Invertebrates also generate lectins, proteins that can bind microbial carbohydrates such as lipopolysaccharides, glucans, mannans, and sialic acid. These include C-type lectins and pentraxins and are thus analogous to mammalian acute-phase proteins. These invertebrate lectins act as opsonins and enhance activation of the proPO system. Insects also produce the antibacterial enzyme lysozyme.

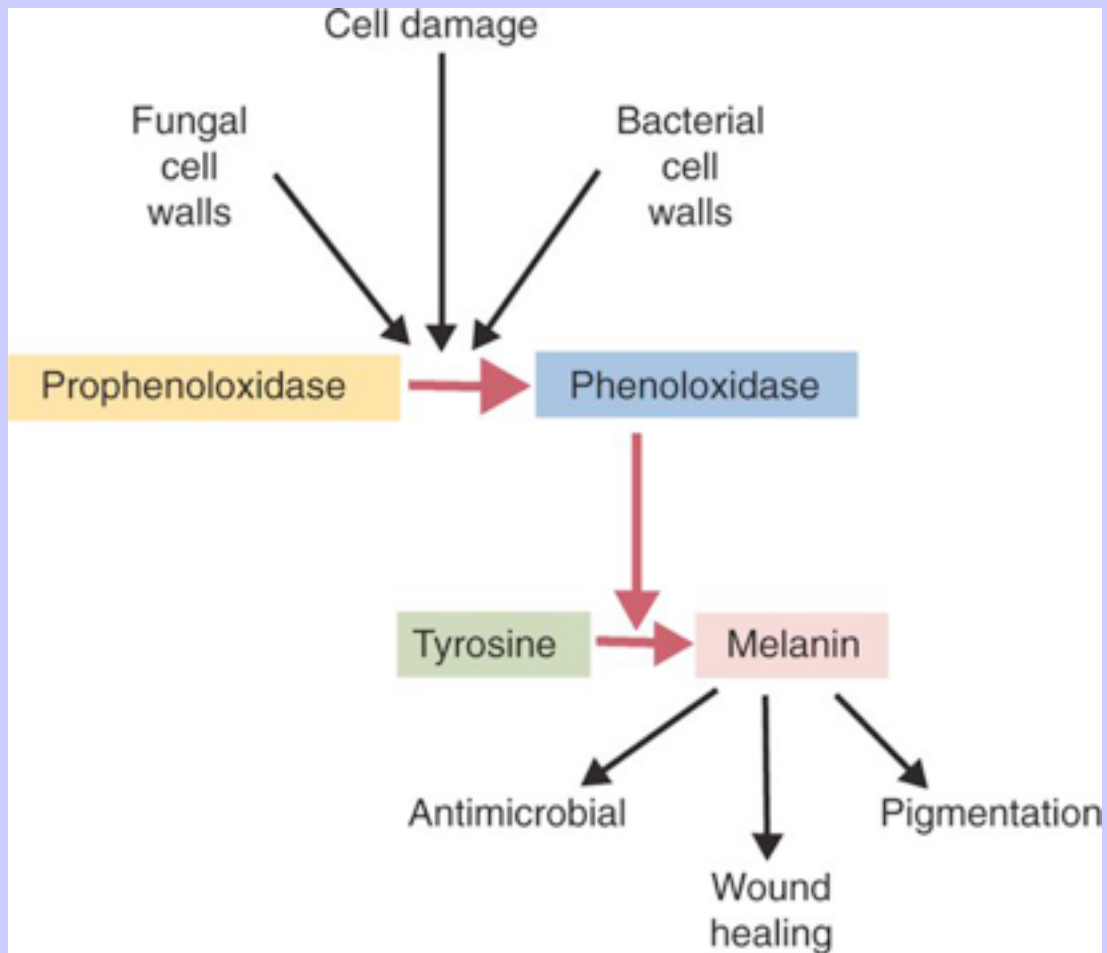
The complement system is very ancient, with some components originating more than 1000 million years ago (Myr ago), long before the emergence of vertebrates. Two complement-like proteins C3 and Bf have been traced back as far as the echinoderms. It is likely that the ancestral C3 was proteolytically activated by Bf and then formed a covalent thioester bond with foreign molecules. When the chordates emerged 900 Myr ago, molecules such as mannose-binding lectin (MBL) and the MBL-associated serine proteases (MASPs) were recruited to the complement system to establish the lectin pathway. Proteins homologous to mammalian MBL and ficolins, two MASPs, C3, C2/factor B, and a C3 receptor have been identified in ascidians (sea squirts). Thus invertebrates have both alternative and lectin pathways. Once activated through these pathways, invertebrate complement can opsonize microbial invaders.

37.2.2.4

RNA Interference

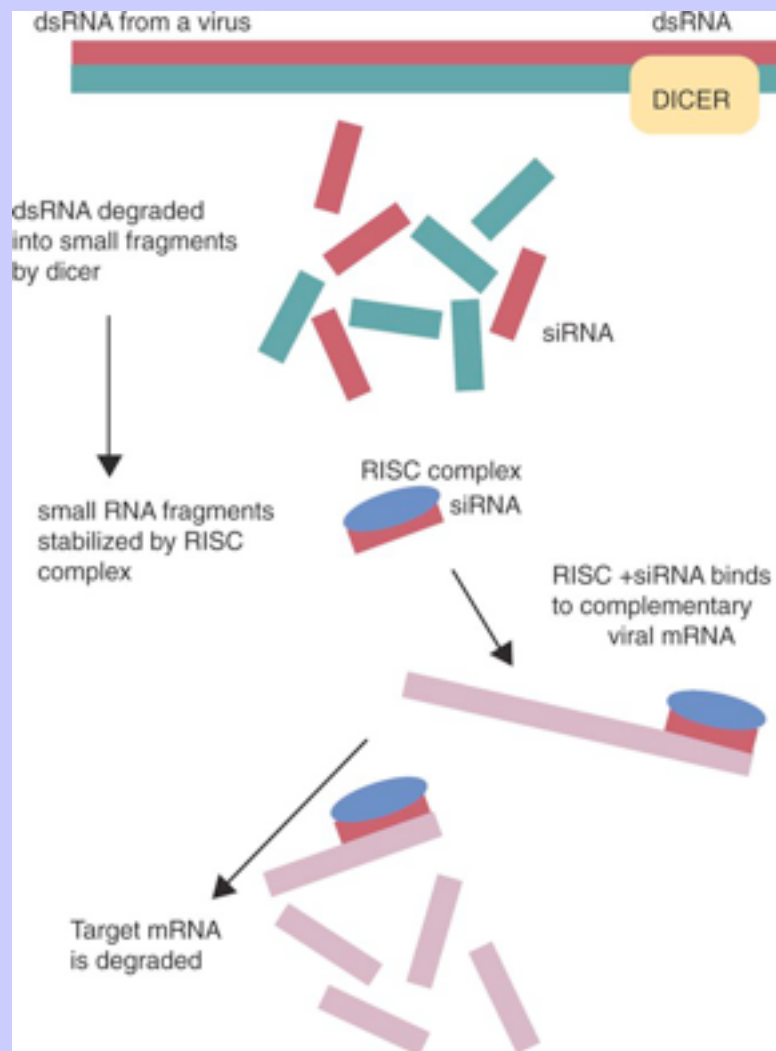
The intracellular RNA interference pathway (RNAi) is a gene-silencing system that appears to have evolved to prevent viruses from replicating within infected cells. It is especially important as a defense system in invertebrates ([Figure 37-3](#)). RNA normally occurs only in a single-stranded form. Long segments of double-stranded RNA (dsRNA) are not present in a healthy eukaryotic cell, but they do occur if a cell is infected by an RNA virus. Therefore, if a virus induces a cell to produce dsRNA, it is rapidly degraded into many short fragments by an enzyme called dicer. These fragments, or small-interfering RNAs (siRNAs), are then stabilized by a set of proteins called the RISC complex. Half of

FIGURE 37-2 The prophenoloxidase pathway is an enzyme cascade system found in many invertebrates, where it serves a key defensive role.



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FIGURE 37-3 The mechanism of RNA interference, an important defensive mechanism in invertebrates (and plants). Double-stranded RNA should not be present in the cytoplasm of normal, healthy cells. Its presence indicates that an RNA virus is infecting a cell. Thus this dsRNA is degraded by an enzyme called dicer into short fragments (short-interfering RNA). The short fragments are then stabilized by a set of proteins called the RISC complex. Half of these siRNA fragments will be complementary to viral mRNAs within the cell. As a result, they will bind specifically to the mRNA. Once this happens the mRNA will be degraded.



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these siRNAs will be complementary to the viral messenger RNAs and as a result can serve as templates for identifying them. Once these target mRNAs are identified by binding to the RISC complex, they are degraded rapidly and viral replication is blocked.

37.2.3 Acquired Immunity

Invertebrates do not make antibodies. The ability to mount adaptive immune responses arose with the jawed vertebrates. Nevertheless, proteins belonging to the immunoglobulin superfamily have been detected in arthropods, echinoderms, and mollusks, as well as in protochordates. Some of these proteins can bind specifically to foreign molecules. Thus in insects, there is a protein member of the immunoglobulin superfamily called Dscam that can be extensively diversified by alternate splicing. Isoforms of Dscam are expressed in immune tissues and secreted as soluble proteins into the hemolymph. *Drosophila* has the potential to express more than 18,000 isoforms of this molecule. Individual hemocytes may express 14 to 50 forms of Dscam that can bind to bacteria and enhance their phagocytosis. It is not known how self-recognition by Dscam is prevented.

37.2.4 Graft Rejection

Invertebrates can reject allografts and xenografts. For example, cell-mediated allograft rejection occurs in sponges, coelenterates, annelids, and echinoderms. Thus when two identical sponge colonies are placed side by side and made to grow in contact with each other, no reaction occurs. If, however, sponges from two different colonies are made to grow in contact, local destruction of tissue occurs along the area of contact as each sponge attempts to destroy the other.

Annelids such as earthworms can reject both allografts and xenografts. The rejection of xenografts (from other species of earthworms) takes about 20 days. Cells invade the graft. The grafted tissue turns white, swells, becomes edematous, and eventually dies. If the recipient worms are grafted with a second piece of skin from the same donor, the second graft is rejected faster than the first. This ability to rapidly reject second grafts may be adoptively transferred by coelomocytes from sensitized animals.

37.3 IMMUNITY IN VERTEBRATES

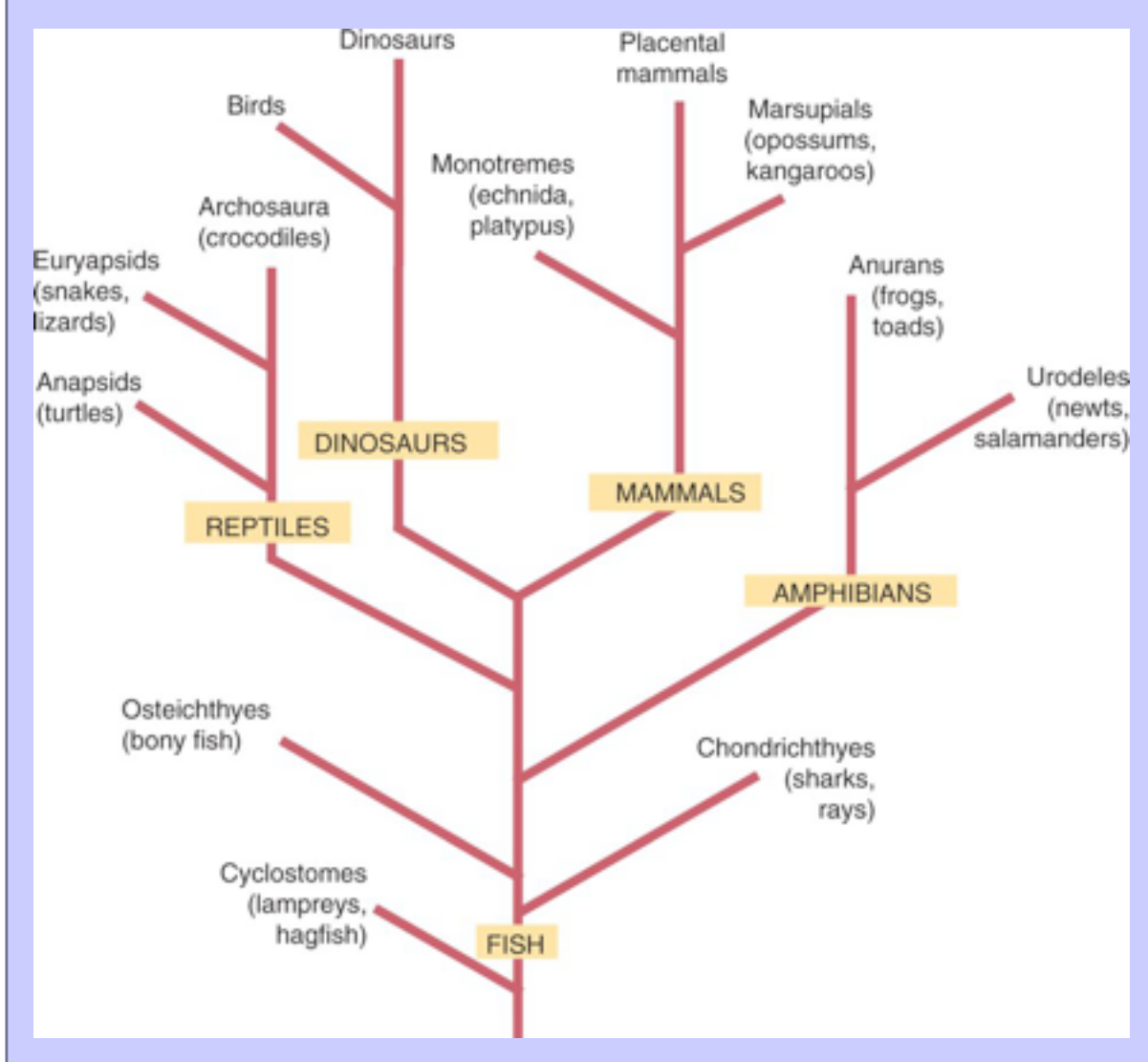
There are seven classes of living vertebrates: the jawless fish, the cartilaginous fish, the bony fish, the amphibians, the reptiles, the birds, and the mammals ([Figure 37-4](#)).

The fish diverged about 450 Myr ago, long before the appearance of the mammals. The least complex living fish belong to the class Agnatha, the jawless fish, or cyclostomes such as the lampreys and the hagfish. Considerably more complex than the cyclostomes are the Chondrichthyes. These are the fish with cartilaginous skeletons and include the rays and sharks (the elasmobranchs). The most complex fish are the bony fish of the class Osteichthyes, which include the overwhelming majority of modern fish, the teleosts. Because they evolved so long ago, fish are much more heterogeneous than mammals, and major differences exist between the immune systems of each class.

There are two major orders of amphibians: the less-evolved Urodela, which includes long-bodied, tailed amphibians such as the salamanders and newts; and the Anura, an advanced, tailless order that includes

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FIGURE 37-4 A simplified phylogenetic tree showing the major relationships among the vertebrates.



the frogs and toads. These also differ significantly in their immune capabilities.

Three subclasses of reptiles currently exist: the Anapsida, which includes the turtles; the Lepidosauria, which consists of the lizards and snakes; and the Archosauria, which includes the crocodiles and alligators.

The dinosaurs, although related to the reptile Archosauria, were sufficiently different from true reptiles to be in a class of their own, the Dinosauria. Although the great majority of dinosaurs disappeared 65 Myr ago at the end of the Cretaceous period, their modern descendants are the birds, the members of the class Aves. Unlike the reptiles, birds are (and dinosaurs probably were) endothermic, or warm blooded. As a result of this, birds share with mammals all the benefits that come from greatly increased physiological and biochemical efficiency.

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The mammals consist of three orders: the prototherians, composed of the monotremes, or egg-laying mammals, such as the platypus and the echidna; the metatherians, composed of the marsupials or pouched mammals, such as the opossum and the kangaroos; and the eutherians, or placental mammals. The marsupials and eutherians are each other's closest relatives. The two groups diverged about 172 Myr ago. The bulk of this book is devoted to the immunology of eutherian mammals.

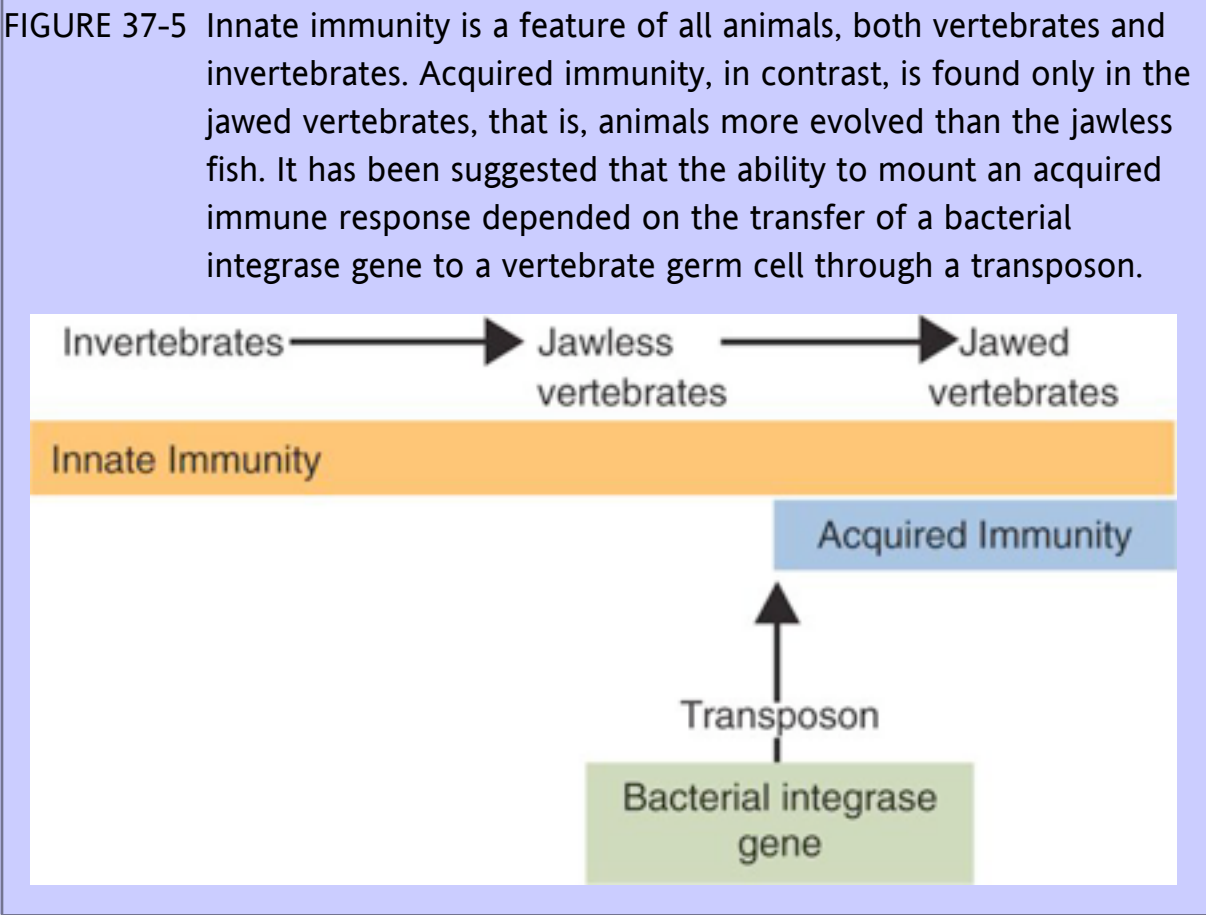
37.4 IMMUNITY IN CYCLOSTOMES

The most primitive of living vertebrates are the cyclostomes, the fish without jaws, including the lampreys and the hagfish. These fish make several different types of proteins that can bind to bacteria and enhance phagocytosis by leukocytes. Some of these proteins are complement-like. Their amino acid sequences resemble C3, C4, and C5, and they contain a hidden thioester bond. Lampreys have an ortholog of mammalian C1q that acts as a lectin. Cyclostomes possess both the alternate and lectin pathways but lack the lytic components of complement. The lamprey complement system thus promotes phagocytosis rather than lysis. Lamprey C3 has features of the common ancestor of mammalian C3 and C4, and lamprey factor B resembles the common ancestor of factor B and C2.

Cyclostomes have two types of blood leukocytes. One population is monocyte-like. The other population

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looks like lymphocytes, and they express variable lymphocyte receptors (VLRs). Although cyclostomes cannot make immunoglobulins, they do generate a great diversity of antigen-binding molecules by re-arranging the DNA

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coding for these VLRs. They generate a highly diverse repertoire of these proteins by inserting leucine-rich modules into an incomplete VLR germline gene. These modules are obtained from a large library located at each end of the VLR gene. They are inserted into the middle of the VLR to generate a functional gene. As a result, the binding site of these VLRs is lined by hypervariable, positively selected amino acids. It is calculated that they may be able to assemble as many as 10^{14} unique receptors in this way. It also appears that each lamprey lymphocyte has a specific VLR, suggesting that clonal selection operates in this system. VLRs are probably anchored to the lymphocyte membrane and released after antigenic stimulation. This therefore represents a very different mechanism from that involving immunoglobulin or TLR diversity, which emerged around the same time. However, the emergence of this different mechanism around 450 Myr ago serves to emphasize yet again the benefits of a lymphocyte-based immune system.

Hagfish kept under good conditions in a warm environment can reject skin allografts. First grafts take about 72 days at 18° C to be rejected; second grafts are rejected in about 28 days. This rejection is presumably due to innate mechanisms.

37.4.1 The Immunological “Big Bang”

The acquired immune system depends on possession of two key antigen receptor systems, the T cell antigen receptor (TCR) and the B cell antigen receptor. Both of these require the rearrangement of V, D, and J gene segments to form functional, antigen-binding receptors. Invertebrates and cyclostomes cannot rearrange these genes, but cartilaginous and bony fish can. Sometime during the 100 million years between the divergence of jawless and jawed vertebrates and the emergence of cartilaginous and bony fish, about 450 Myr ago, the enzymic machinery needed for the recombination of V gene segments emerged. The mechanism of this sudden appearance is unknown. It has been suggested, however, that a transposon carrying the precursors of the recombinase-activating genes (RAG-1 and RAG-2; most likely a bacterial integrase) was successfully inserted into an immunoglobulin superfamily V-like gene within the germ line of the early jawed vertebrates ([Figure 37-5](#)). As a result, the immunoglobulin gene could be expressed only after splicing mediated by the RAG enzymes. Thus emerged, in a major evolutionary leap, the ability to generate antigen-binding sites and functional immunoglobulins. This, for the first time, permitted animals to respond specifically to previously encountered antigens. The advantages of this new “improved” system were such that it is now a feature of all jawed vertebrates. This did not, of course, result in discarding of the innate immune defenses. Thus lectins, the complement system, and the natural killer (NK) cell system remain essential components of vertebrate immunity. It is also important to point out that acquired immunity did not destroy infectious agents. It simply made life more difficult for them and so conferred an incremental selective advantage to animals with such defenses. Evolution is, however, short-sighted. The selective advantage of acquired immunity came with attendant costs—the potential for autoimmune disease.

37.5 IMMUNITY IN JAWED FISH

37.5.1 Innate Immunity

Phagocytosis in fish is similar to that described for mammals. For example, fish granulocytes enter inflammatory sites first and their numbers peak in about 12 to 24 hours. This is followed by a later wave of macrophages and possibly lymphocytes. The granulocytes are attracted by microbial products and soluble tissue mediators. The response tends to be prolonged, and macrophage numbers peak at 2 to 7 days. In fish, granulocytes originate from the anterior kidney, whereas the macrophages develop from blood monocytes. Fish macrophages are found in many sites, especially the mesentery, splenic ellipsoids, kidney, and atrium of the heart.

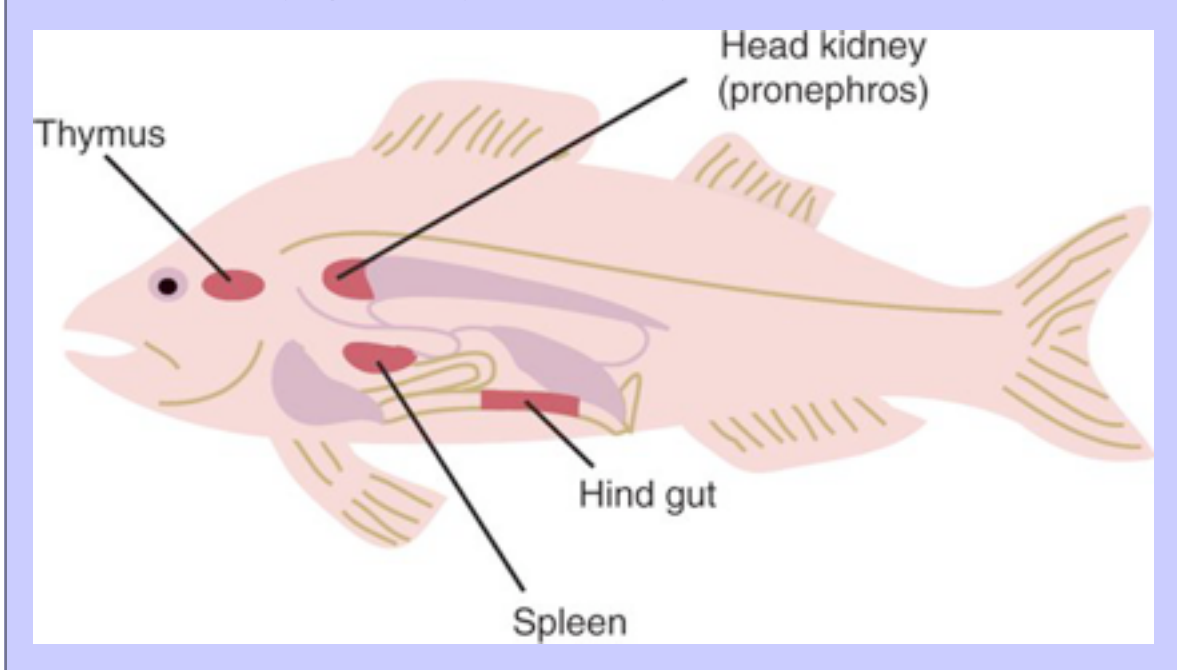
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Teleost neutrophils are similar in morphology and probably function to mammalian neutrophils and are frequently seen in inflammatory lesions. These neutrophils are phagocytic, and their numbers increase in response to infections. They possess most of the

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FIGURE 37-6 The lymphoid organs of a bony fish.



enzymes of mammalian neutrophils. It has been suggested that in some species neutrophils may carry out their bactericidal function extracellularly rather than intracellularly. The release of oxidants from neutrophils at inflammatory sites may cause severe tissue damage. The fat of fish is highly unsaturated as an adaptation to low temperatures. Polyunsaturated fats are prone to oxidation, and free radicals may therefore oxidize tissue lipids. Fish, therefore, require a powerful means of modulating this response. The brown pigment melanin can quench free radicals, and melanin-containing cells are common in the lymphoid tissues of most bony fish, as well as in inflammatory lesions. It probably protects tissues against oxidants produced by phagocytic cells.

Both bony and cartilaginous fish can produce lysozyme, lectins, defensins, complement, and acute-phase proteins. Lysozyme is present in fish eggs and may protect the developing embryo. This fish lysozyme is much more broadly reactive than the mammalian enzyme and is active against both Gram-positive and Gram-negative bacteria. Fish acute-phase proteins include C-reactive protein, serum amyloid A, and serum amyloid P. However, their rise is much less pronounced than in mammals. An MBL has been identified in species such as the Atlantic salmon. Natural cytotoxic cells similar to mammalian NK cells have been described in bony fish. They are produced in the anterior kidney.

Cartilaginous and bony fish possess all three complement pathways: namely, the classical, alternate, and lectin activator pathways. The gene duplications required for the development of the classical pathway appeared prior to the appearance of cartilaginous fish. The fish lytic pathway generates a membrane attack complex similar to that formed in mammals, although it works at a lower optimum temperature ($\approx 25^{\circ}\text{C}$). Unlike other vertebrates, in which C3 is coded for by a single copy gene, in bony fish, C3 is produced in multiple functional isoforms. Thus rainbow trout have four C3 isoforms, carp have eight, and sea bream have five. They differ in their structure and in their ability to bind to different activating surfaces. It has been suggested that this complement

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polymorphism permits the most effective destruction of different invading microorganisms. As in mammals, C3 is the complement component at highest concentration in fish serum. Teleosts may have multiple isoforms of C4 as well. Regulatory proteins similar to C4-binding protein and factor H have been identified in sand bass.

Fish TLRs are similar to those found in mammals. There are six major families of vertebrate TLRs; within each family, the TLRs recognize a general class of PAMP. The functions and binding specificities of each TLR family have remained unchanged as the vertebrates evolved. (The microbes haven't changed, so neither have the TLRs.)

37.5.2

Acquired Immunity

Both cartilaginous and bony fish can mount acquired immune responses and have a complete set of lymphoid organs except for a bone marrow ([Figure 37-6](#)). Thus they have a thymus located just above the pharynx. It arises from the first gill arches. In immature fish small pores lead from the pharynx to the thymus, suggesting that it may be stimulated directly by antigens in the surrounding water. Thymectomy in fish can lead to prolongation of allograft survival and reduced antibody responses. Antibodies or antigen-binding cells may be detected in the thymus during an immune response, suggesting that it contains both T- and B-like cells. Although the thymus may involute in response to hormones or season, age involution is inconsistent and the thymus may be found in many older fish.

The kidneys of fish differentiate into two sections. The opisthonephros, or posterior kidney, is an excretory organ that serves the same function as the mammalian kidney. In contrast, the pronephros, or anterior kidney, is a lymphoid organ containing antibody-forming cells and phagocytes. It thus performs a function analogous to mammalian bone marrow and lymph nodes. Fish have a spleen whose structure and location are similar to those in mammals.

Aggregates of lymphocytes are prominent in the fish intestinal tract. In addition, lymphomyeloid structures that appear to produce granulocytes are found in the submucosa of the esophagus (Leydig organ) and in the gonads (epigonal organ) of sharks. Some species possess both, but others may have only one of these organs. In cartilaginous fish the epigonal organ and the Leydig organ express RAG proteins, as well as TdT (see [Chapter 12](#)) and other B cell-specific transcription factors and appear to be primary lymphoid organs. The epigonal organ appears to function, like mammalian bone marrow, as a source of B cells throughout life.

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Fish possess aggregations of macrophages that contain pigments such as melanin and hemosiderin. These melanomacrophage centers are found in the spleen, liver, and kidney. Antigens may persist in these centers for long periods, and they appear to be precursors of the germinal centers found in more evolved vertebrates.

Fish possess true lymphocytes that resemble those described in mammals. Thus B cells can be found in the thymus, anterior kidney, spleen, Leydig organ, and blood, and their surface membrane immuno-globulin acts as an antigen receptor. These B cells can mature into plasma cells. Unlike mammalian B cells, however, B cells from teleost fish can phagocytose particles, generate phagolysosomes, and kill ingested microbes. These findings support the idea that B cells may have evolved from an ancestral phagocytic cell and may account for the apparent similarities between macrophages and mammalian B-1 cells. Both helper and cytotoxic T cells can be detected in fish.

37.5.2.1

Immunoglobulins

The cartilaginous fish such as the sharks are the least-evolved vertebrates known to possess an acquired immune system. Like other species, they produce a diversity of immunoglobulin isotypes.

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All vertebrate light chains can be classified into one of four ancestral “clans” that originated before the emergence of cartilaginous fish: one restricted to elasmobranchs, called s-cart; one in all cold-blooded vertebrates, called s; one in all groups except birds (k); and one in all groups except bony fish (λ). All four have maintained separate identities since their emergence 450 Myr ago, suggesting that there must be a functional basis for their differences.

Immunoglobulins are seen for the first time in cartilaginous fish because they possess the recombinant activator genes RAG-1 and RAG-2. The manner in which these immunoglobulin molecules are coded for by light and heavy chain genes and the structures of the V, J, and C gene segments are similar to those seen in mammals. Nevertheless, they differ from mammals in the organization of immunoglobulin gene segments within the genome. For example, sharks and other elasmobranch fish have clustered immunoglobulin genes, where V, D, J, and C segments form clusters that are duplicated many times; thus:

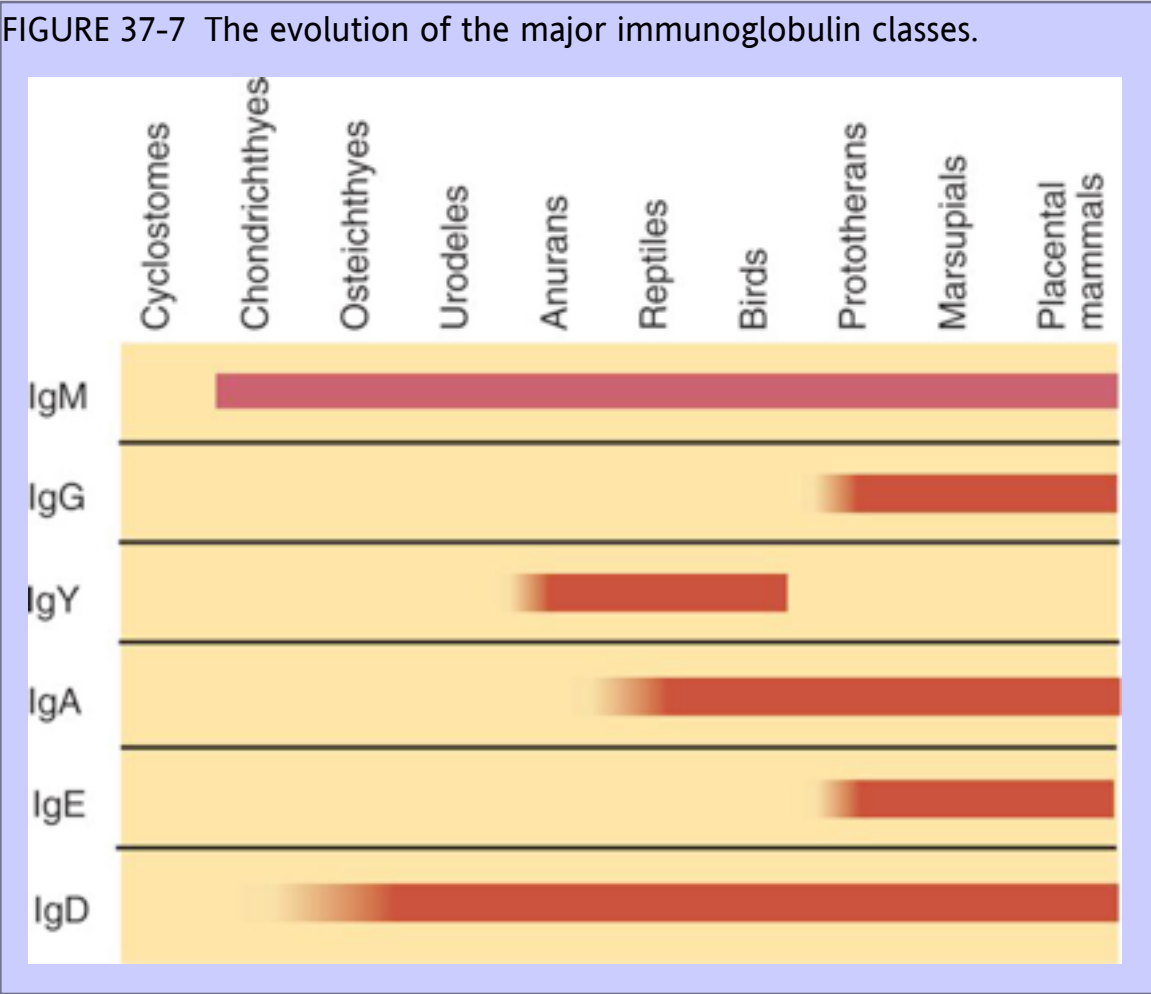
-VDJC—VDJC—VDJC—VDJC—VDJC—

There are 200 to 500 of these VDJC clusters in sharks; each cluster is about 16 kilobases in size. About half of these clusters appear to be functional. (This arrangement is somewhat similar to that seen in the TCR- α , and TCR- β genes in mammals.) Teleost fish, in contrast, have an immunoglobulin heavy chain gene arrangement similar to that of mammals (the translocon pattern), with multiple Vh genes arranged thus:

-V-V-V-V-V-V-D-D-J-J-J-J-C—

Teleost light chain genes, however, are arranged in the clustered pattern. Thus, for example, in catfish, heavy chains are constructed in the translocon pattern, and light chain genes are in the clustered pattern. Shark immunoglobulins show evidence of somatic hypermutation.

Immunoglobulin M (IgM) is the most ancient of the immunoglobulin classes and is found in both bony and cartilaginous fish ([Figure 37-7](#)). Cartilaginous fish usually have both pentameric and monomeric serum IgM. Bony fish have tetrameric and monomeric IgM. These different forms may compensate for a lack of IgG. Recently, several additional isotypes have been identified in elasmobranchs. These include IgNAR (new antigen receptor) in the nurse shark, IgW in the sandbar shark, and IgR in the skate. IgNAR consists only of heavy chains with no associated light chains. IgNAR sequences in young sharks are infrequently mutated, but mutation increases significantly as the fish mature. Antigens bind to the single heavy chain variable domain in a manner similar to camel antibodies. X-ray crystallography shows that these variable domains have only two complementarity-determining regions (CDRs). The molecules also possess a vestigial CDR at the other end of the heavy chain at a location



associated with cell adhesion. Thus it is suggested that these primitive immunoglobulins may have originated as cell adhesion molecules.

Trout also possess an immunoglobulin isotype distinct from IgG and IgM. This unique isotype, called (unfortunately) IgT, shares VL with the other isotypes but has its own exclusive set of VH and JH genes. The IgT D, J, and C gene segments are located upstream of the IgM genes, a very unusual arrangement. As might be expected, IgT is produced very early in the life of the developing trout embryo.

The heavy chain of IgW contains six C_H domains, two more than IgM. Sequence analysis indicates that it is orthologous to IgD. The IgW isotype occurs in two forms: a conventional form and a short, truncated form similar to the truncated form of avian IgY. IgR, on the other hand, has only two C_H domains. Peptides homologous to the J chain have been described in some fish but not in others. In the absence of J chains, the IgM monomers are held together by noncovalent bonds. IgD genes and products have been identified in catfish, halibut, salmon, and cod. They have some similarities to mammalian IgD, including coexpression with IgM on the B cell surface as a result of alternative splicing. The function of teleost IgD is unknown. Light chain isotypes have been described in catfish, salmon, and trout. Two different light chains have been described, and none is closely homologous to mammalian κ and λ chains. Fish antibody responses are characterized by the predominance of IgM and by their relatively poor secondary responses.

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An unusual feature of elasmobranch immunity is the existence of immunoglobulin genes rearranged in the germ line. These appear to play a role early in development but tend to be silenced in later life, when their function is replaced by rearranging genes. Thus in the nurse shark, the predominant immunoglobulin in neonates is coded for by a germ line–rearranged gene. This might be a transitional stage between innate and acquired immunity.

In the presence of normal serum as a source of complement, fish antibodies can lyse target cells. Likewise, fish antibodies are effective at agglutination. There is no evidence that fish antibodies can function as opsonins, nor have Fc receptors been detected on fish phagocytic cells. The blood vessel walls of fish are permeable to IgM. As a result, antibodies are found in most tissue fluids (plasma, lymph, skin mucus). Transfer of antibodies from immunized females to their eggs has been described in the plaice.

Not all antigens are effective immunogens in fish. Soluble protein antigens are poorly immunogenic, in contrast to particulate antigens such as bacteria or foreign erythrocytes that are highly immunogenic. Many cartilaginous fish show seasonal effects on antibody production; that is, under constant conditions of light and temperature, immune responses are poorer in winter than in summer. Social interactions can also influence their immune response: Fish kept at high population density are immunosuppressed.

37.5.2.2

Cell-Mediated Immunity

The acquisition of recombinase activity enabled fish to generate rearranged TCRs, and TCR homologs have been identified in both elasmobranchs and teleosts. Their overall structure is similar to that in mammals. The germ line TCR genes are not rearranged and are organized in the cluster pattern. Fish have both major histocompatibility complex (MHC) class I and II genes, but these have never been found in agnathans or invertebrates. (MHC class III genes, in contrast, have been found in primitive chordates.) The basic structure of each MHC molecule has been conserved, as has the organization of the class I and class II genes. In teleost fish, however, class I and II loci are on different chromosomes.

Cartilaginous fish reject scale allografts slowly, whereas bony fish reject them much more rapidly. Repeated grafting leads to accelerated rejection. The rejected allografts are infiltrated by lymphocytes and show destruction of blood vessels and pigment cells. As in all ectotherms, graft rejection is slower at lower temperatures. Many different cytokines have been identified in fish, including IL-1, IL-2, IL-3, IL-6, TNF- α , transforming growth factor- β , interferon- β (IFN- β), and IFN- γ .

37.6

IMMUNITY IN AMPHIBIANS

As vertebrates have evolved, they have shown a progressive increase in the complexity of their immune systems ([Figure 37-8](#)). This is well seen in amphibians, in which there are marked differences between the less complex urodeles (tailed amphibians such as the newts and salamanders) and the much more evolved anurans such as the frogs and toads. In addition, amphibians go through a complex metamorphosis as they change from the tadpole into an adult form. This has significant effects on the development of the immune system.

Amphibians have effective innate defenses. A notable feature of their innate immunity is the presence of very potent antimicrobial peptides in the skin. Amphibians also possess a complement system that, although similar to that of mammals, is more effective at 16° C.

37.6.1

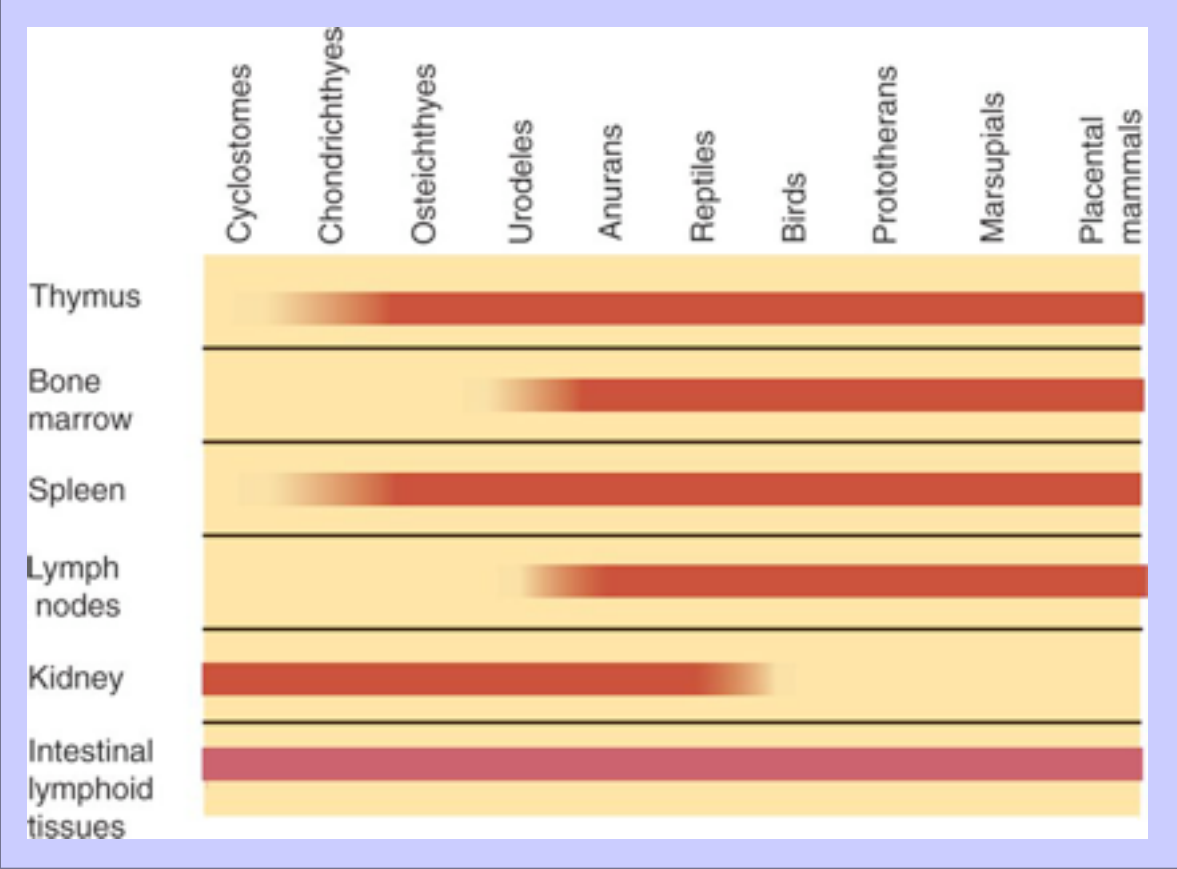
Urodele Amphibians

Urodeles generally lack a bone marrow, although some salamanders may have a small amount of lymphoid

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FIGURE 37-8 The evolution of the major lymphoid organs in vertebrates.



tissue within their long bones. They have a thymus that develops slowly, appearing only at the seventh week of life. Thymectomy delays or blocks rejection of skin allografts. The thymus of urodeles is not divided into cortex and medulla. The kidney retains its lymphoid function, as in fish. Stem cells arise from the intertubular areas of the kidney in both urodeles and anurans. In the spleen the red and white pulps are not separate.

Urodeles produce a monomeric IgM and can mount a good but slow antibody response against bacterial antigens. They do not respond to soluble protein antigens such as serum albumin or ferritin.

It takes about 28 to 42 days for a skin allograft to be rejected in urodele amphibians. The allograft looks healthy for about 3 weeks, and it is then slowly rejected. The rejection of the graft is readily visible because pigment cell destruction turns the skin white. Second-set rejection takes about 8 to 20 days in the newt, and the alloimmune memory lasts at least 90 days.

37.6.2 Anuran Amphibians

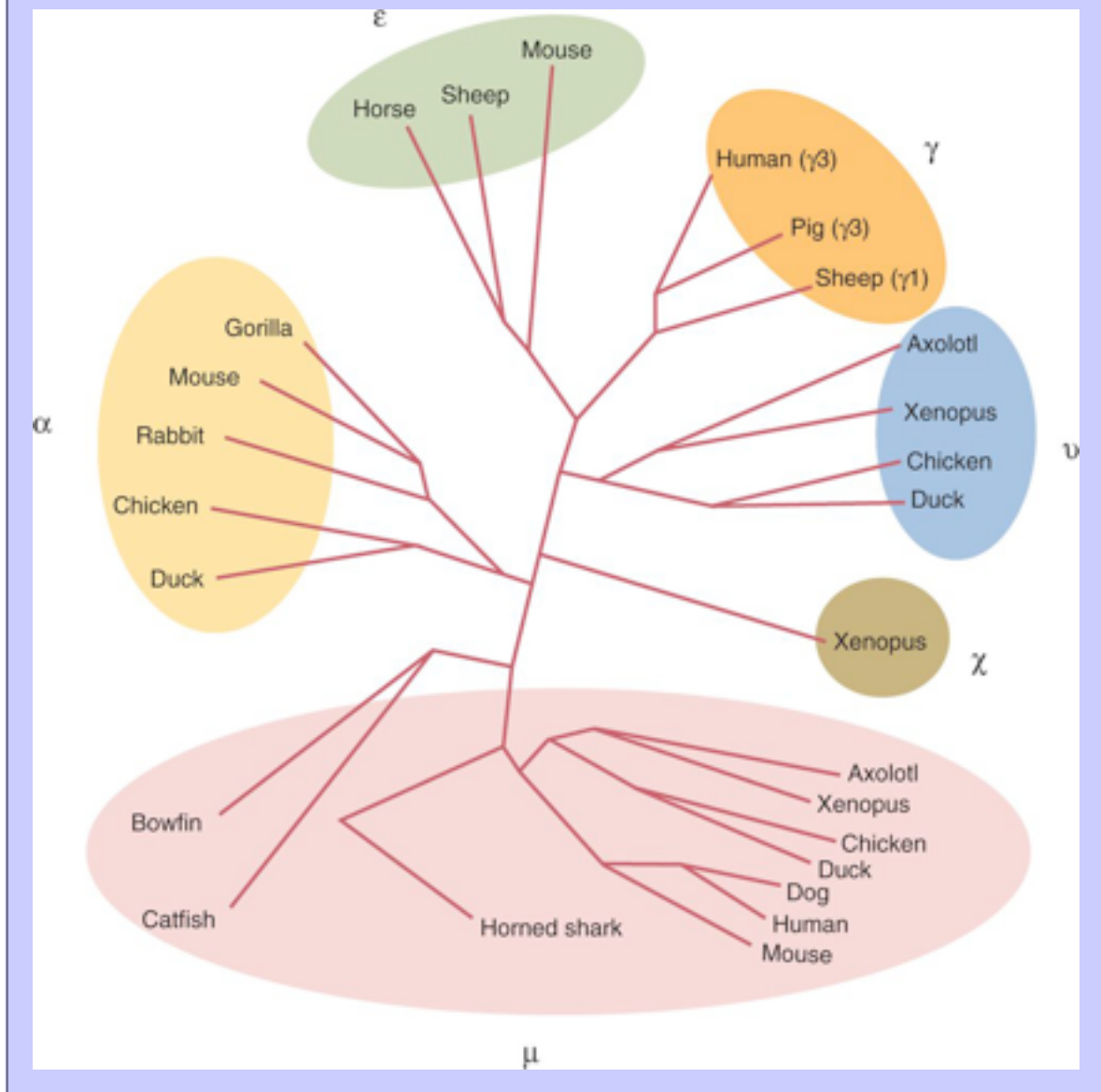
In contrast to urodeles, a fully functional bone marrow is present in anurans (frogs and toads). Their thymus arises from the second pharyngeal pouches and involutes by about 1 year of age. It also involutes during metamorphosis from the tadpole to the adult stage and then rapidly regenerates. The thymus lies just below the skin posterior to the middle ear. In contrast to the thymus of the fish, it shows a distinct separation between the outer cortex and central medulla. The thymic cortex is full of proliferating lymphocytes. The medulla contains fewer lymphocytes, but thymic corpuscles are present. Immunoglobulins can be found on about 80% of these thymocytes. Larval thymectomy in the toad reduces the response to foreign red blood cells, but the response to bacterial lipopolysaccharide is unaffected, suggesting this is a T-independent response. Thymectomy also slows but does not completely prevent allograft rejection in toads. Some residual T cell function redevelops several months after larval thymectomy, suggesting that extrathymic T cell development may occur. In frogs and toads, for the first time, boundary layer cells separate the red pulp and the periarteriolar white pulp of the spleen. Structures that resemble lymph nodes are seen in some anuran amphibians. These proto-lymph nodes consist of a mass of lymphocytes surrounding blood sinusoids. As a result, they filter blood rather than lymph. Nodular lymphoid aggregates do not seem to be present in the intestine of urodeles but are seen in anurans.

In their branchial region, larval anurans such as the bullfrog tadpole have lymphomyeloid organs called ventral cavity bodies. Sinusoids in these organs are lined with macrophages that effectively remove injected particulate antigens from the blood. Removal of these organs renders tadpoles incapable of making antibodies to soluble antigens. They disappear at metamorphosis. Lymphocytes are found in large numbers in the subcapsular region of the liver in fish, amphibians, and reptiles. These lymphocyte accumulations occur close to blood sinuses and may have a stem cell function.

Both adult and larval amphibians have circulating B and T cells. They probably originate in the ventral cavity bodies or the liver. The thymic lymphocytes and about 80% of circulating lymphocytes carry surface IgM. Frogs possess NK-like and T cytotoxic-like killer cells.

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FIGURE 37-9 The evolutionary relationships among the major vertebrate immunoglobulin heavy chains. This is a distance tree constructed by aligning the amino acid sequences of representative vertebrate immunoglobulin H-chain constant regions. (From Warr GW, Magor KE, Higgins DA: *Immunol Today* 16:392-398, 1995.)



Anuran amphibians have two or three immuno-globulin classes and are the least evolved verte-brates to show isotype switching. Their IgM consists of either pentamers or hexamers (*in Xenopus*) and one or two of the low-molecular weight molecules IgY (with a 66 kDa ν heavy chain) and IgX (with a 64 kDa χ heavy chain). IgX is a

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distinctly different immunoglobulin class not found in other vertebrates ([Figure 37-9](#)). *Xenopus* immunoglobulins also contain two types of light chain, and anuran amphibians possess secretory immunoglobulins in bile and the intestine (but not in skin mucus). These consist of IgM and IgY but not IgA. In the axolotl (*Ambystoma mexicanum*) IgY is a secretory immunoglobulin found in close association with secretory component-like molecules. This is different from *Xenopus*, in which IgY behaves like avian IgY or mammalian IgG. Amphibian antibody diversity is generated in a fashion similar to that in mammals.

The gene for the IgD heavy chain (d) has been identified in the toad *Xenopus tropicalis*, where it is expressed on the surface of mature B cells. The location of the Cδ heavy chain is the same as that found in mammals, immediately 3' to the IgM gene. Sequence analysis, however, shows that *Xenopus* IgD is orthologous to IgW found only in cartilaginous fish and lungfish. This implies that IgD/W was present in these ancestors of all living jawed vertebrates. In contrast to IgM, IgD is structurally highly variable. Thus in different species it shows many duplications and deletions of domains, the presence of multiple splice forms, or even the loss of the entire gene as in birds (chickens?). As a result, it probably plays different roles in different vertebrate taxa. An additional isotype, IgF with a Cφ heavy chain, has also been identified in *Xenopus*. It is unique in having a hinge region. This is the earliest example of such a structure. The IGH locus of *X. tropicalis* therefore has the following order, 5'-V_H-D_H-J_H-C_m-C_d-C_c-C_u-C_f-3'.

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Frogs given bacteria or foreign erythrocytes will produce only IgM. Bacteriophages or soluble foreign proteins induce both IgM and IgY. Soluble antigens and bacteriophage can induce the production of both IgY and IgM in adult toads. The IgY takes up to a month to appear, and its level is very low. Anuran larvae will make only IgM antibodies unless immunized several times, when low levels of IgY are produced. Amphibians do not mount a secondary immune response to erythrocytes and bacteria, but memory develops in response to the antigens that stimulate an IgY response. Studies on immunological memory are complicated by the fact that antigens may persist in the circulation for several months following injection. Anaphylaxis-like reactions have been described in amphibians and reptiles.

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Amphibians have T cells with functional TCRs, and anurans such as bullfrogs and toads show a fairly rapid allograft rejection. A first-set reaction takes about 14 days at 25° C. The graft shows capillary dilation, lymphocyte infiltration, and disintegration of pigment cells. Second allografts do not even become vascularized and are destroyed within a few days. If these amphibians are kept in the cold, a skin allograft may take as long as 200 days to be rejected. Delayed hypersensitivity reactions have been described in the axolotl (*Ambystoma*) and *Xenopus* in response to mycobacterial sensitization.

During amphibian metamorphosis from larval stage to adult, there is a temporary immunosuppression as shown by slowing of allograft rejection. Some allografts may even be tolerated at this time. As tadpoles change into frogs or toads, the thymus shrinks and there is a drop in the numbers of B cells and antibody levels.

Cytokines identified in amphibians include IL-1, IL-2, and the interferons. *Xenopus* lymphocytes possess a receptor resembling IL-2R and can be stimulated by human IL-2 in vivo. *Xenopus* peritoneal cells generate IL-1-like activity.

Xenopus has a well-characterized MHC with class I, II, and III regions called XLA. The class II region contains genes for both α and β chains. These are 30 to 35 kDa transmembrane glycoproteins. About 20 class I and 30 class II alleles are believed to exist. The class III region contains a gene for C4. It is interesting to note that although MHC class II molecules are expressed early in larval development on B cells and tadpole epithelia, MHC class I molecules are not expressed before larval metamorphosis.

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37.7 IMMUNITY IN REPTILES

The reptilian thymus develops from the pharyngeal pouches and is structurally similar to that seen in other classes of vertebrates. Both age and seasonal involution of the reptile thymus have been reported. The thymus shrinks in winter and enlarges in summer. The reptilian spleen usually shows a clear separation between red and white pulps.

Lymphomyeloid nodes that resemble lymph nodes are seen in reptiles. They have a simple structure consisting of a lymphoid parenchyma with phagocytes and intervening sinusoids. Primitive lymph nodules surrounding the aorta, vena cava, and jugular veins are also found. Lymphocytes and plasma cells are found in nodules in the intestinal wall of all the more evolved vertebrates. Some turtles and snakes (but not alligators) have lymphoid aggregations that project into the cloacal lumen, called the cloacal complex. These aggregates are larger in adults than in young turtles and are therefore not primary lymphoid organs and cannot be regarded as a primitive bursa. A few lymphocytes are found in the kidney of reptiles.

The reptiles that have been studied possess both IgM and IgY. The IgM of turtles is comparable to mammalian IgM in size, chain structure, and carbohydrate content. The IgY is found in both the full-sized and truncated isoforms (although some turtles may have only the truncated isoform). Geckos (*Eublepharis sp.*) produce a form of IgA. Sequence analysis shows that while its C_H1 and C_H2 domains are homologous to Xenopus IgY, its C_H3 and C_H4 domains are homologous to Xenopus IgM. It appears therefore that recombination between IgY and IgM genes gave rise to this IgA. Alligators possess two different forms of immunoglobulin light chain, perhaps homologs of mammalian κ and λ .

Three C3 genes are present in the cobra. One codes for functional C3 in serum. The other two are expressed only in the venom gland and encode a C3c-like molecule present in venom that forms a stable C3-convertase in the presence of factor B.

Turtles and lizards immunized with bovine serum albumin, pig serum, or red blood cells can mount both primary and secondary antibody responses. The antibody produced in the primary response is IgM; the antibody produced in the secondary response is IgY. All reptile antibody responses appear to be T dependent. Secondary responses and IgY antibody production do not occur in response to certain bacterial antigens such as *Salmonella* serovar *adelaide*, *Brucella abortus*, or *Salmonella* serovar *typhimurium*. The reader may recollect that a similar situation occurs in mammals, in that thymus-independent antigens such as *Escherichia coli* lipopolysaccharide induce a prolonged IgM response that is distinctly different from that induced by soluble protein antigens (see [Chapter 17](#)).

As in other ectotherms, the rate of allograft rejection is temperature-dependent. Turtles, snakes, and lizards reject allogeneic skin grafts in about 40 days at 25° C. Graft-versus-host disease can be induced by injection of cells from their parents into newborn turtles and can lead to death. The severity of the disease depends on the genetic disparity between the turtles. Mortality, however, is greater at 30° C than at 20° C. Other evidence of cell-mediated immune responses such as mixed lymphocyte reactions and delayed hypersensitivity reactions have been demonstrated in reptiles.

37.8 IMMUNITY IN BIRDS

It must be recognized that the vast majority of studies on the avian immune system have focused on chickens. Thus the statements below, while generally true of chickens, may not necessarily apply to all of the other approximately 9000 bird species. The birds diverged from the mammalian line about 300 Myr ago, which has provided ample opportunity for the immune systems of mammals and birds to evolve major differences.

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Analysis of the complete chicken genome provides some interesting insights into the evolution of the immune system in this species. For example, it has proved possible to identify chicken orthologs of several immune-related genes that were previously believed to be confined to mammals. These include cathelicidin, colony-stimulating factors, and ILs 3, 4, 7, 9, 13, and 26. Chickens have TLR1, 2, 3, 4, 5, and 7 but not TLR8, 9, or 10. It is also of interest to note that some gene families are found in chickens to a much greater extent than in humans. Many of these have roles in immunity and host defense. They include some immunoglobulin receptors, MHC class I molecules, NK cell receptors, and T cell antigens. The significance of this expansion is unclear, but it may simply reflect the different histories of exposure to infectious disease encountered in chickens and humans.

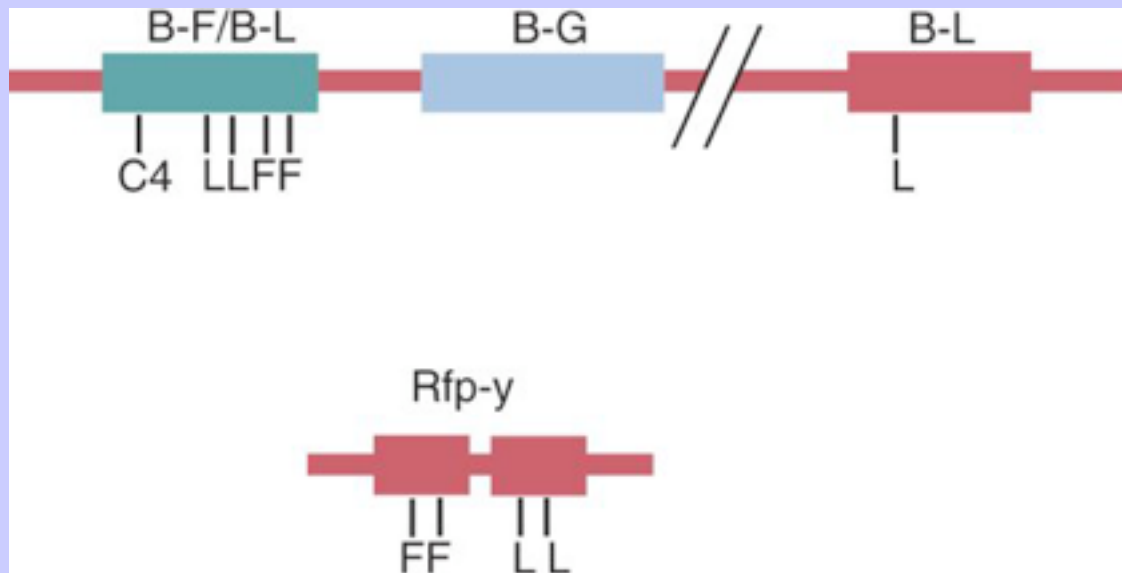
37.8.1

Avian MHC Molecules

The chicken MHC occupies 92 kb, contains only 19 genes, and so is very much smaller and simpler than mammalian (or other avian) MHCs. It is divided into two independent regions designated B and Y. Both are located on microchromosome 16, but they are separated by the nucleolar organizer region ([Figure 37-10](#)). The B region contains three gene clusters. The Y region contains two. Each region contains both class I and class II loci. However, these genes differ significantly between birds and mammals. For example, chickens have DM but not DP, DQ, or DR. They also lack most class III region genes. The B region is also organized differently. Chickens possess a single dominantly expressed class I gene that determines the immune response to infectious pathogens. This is in contrast to most mammals, where immune responsiveness is determined by multiple polymorphic genes. Cluster 1 or the B-F/B-L region contains two class Ia α -chain genes (*B-F*), a C4 gene, and two class II β -chain genes (*B-L*). There is a single class II α -chain gene located about 5 cM away from the β -chain gene. Two clusters (V and VI) form the *B-G*, or class IV, region. These encode blood group antigens. The *B-G* gene products are membrane proteins with molecular weights ranging from 40 to 48 kDa. These molecules can form monomers, homodimers, and heterodimers and are mainly found on red cells and thrombocytes. Related *B-G* molecules are found at low levels on lymphocytes. Their function is unknown. There are two gene clusters in the Y region containing two MHC class I and two MHC class II loci. They differ from the B-region loci in that their products are not expressed on red cells. Genes within the Y region also regulate NK cell recognition.

In the common chicken haplotypes, only a single class I and a single class II molecule are dominantly expressed. Since viruses contain relatively few proteins, MHC-dependent disease susceptibility depends on virus antigens binding to the dominant MHC class I molecule. As a result, possession of specific haplotypes determines disease susceptibility. For example, the haplotype B²¹ is associated with resistance to Marek's disease whereas the B¹⁹ haplotype is associated with susceptibility. Chickens homozygous for B¹ generally have high adult mortality, are highly susceptible to Marek's disease, and respond poorly to *Salmo*

FIGURE 37-10 Structure of the chicken B region (*top*) and the Y region (*bottom*). F genes are class II genes, and L genes are class I genes.



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nella serovar *pullorum* or to human serum albumin. Birds homozygous for B⁵ are able to mount a better antibody response and develop less severe lesions in response to infection with *Eimeria tenella* than B² homozygous birds. Certain MHC genotypes (B^{A4/A4} and B^{A12/A12}) are significantly overrepresented in birds suffering from bacterial arthritis and osteomyelitis primarily caused by *Staphylococcus aureus*.

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The thymus in birds and in primitive mammals is similar to that seen in eutherian mammals. Germinal centers are not seen in fish, amphibian, or reptile spleens. In contrast, the germinal centers of bird lymphoid organs are large and well defined. Although birds are commonly considered not to possess lymph nodes, they do possess structures that can be considered to be their functional equivalent. These avian lymph nodes consist of a central sinus that is the main lumen of a lymphatic vessel. It is surrounded by a sheath of lymphoid tissue that contains germinal centers ([Figure 37-11](#)). Avian lymph nodes have no external capsule.

The bursa of Fabricius has been described in [Chapter 10](#). Bursectomy results in a loss of antibody production, although bursectomized birds can still reject skin allografts. These results have been interpreted to suggest that the bursa is a primary lymphoid organ whose function is to serve as a maturation and differentiation site for the cells of the antibody-forming system. The bursa, however, contains some T cells; it can trap antigens and undertake some antibody synthesis. Birds also have large numbers of lymphocytes in the cecal tonsils and in the skin.

Bird lymphocytes originate in the yoke sac and migrate either to the bursa or to the thymus. Immature lymphocytes that enter the thymus mature under the influence of factors derived from thymic epithelial cells, and cells with recognizable T cell markers emigrate from the thymus. T cells constitute between 60% and 70% of blood lymphocytes.

37.8.2 Immunoglobulin Classes

There are three principal immunoglobulin classes in birds (chickens): IgY, IgM, and IgA. No avian IgD gene has yet been identified.

37.8.2.1 Immunoglobulin Y

The principal immunoglobulin in chicken serum is called IgY. Although somewhat similar to mammalian IgG, it has sufficient molecular differences to warrant a different designation. Some investigators have reported the existence of three immunoglobulin subclasses—termed IgY1, IgY2, and IgY3—although this has not been completely substantiated.

Like the immunoglobulins of mammals, IgY consists of two heavy and two light chains ([Figure 37-12](#)). The heavy chains, called epsilon (ν) chains, usually consist of one variable and four constant domains, and the complete molecule has a molecular weight of about 180 kDa (7.8 S). However, some birds have a truncated isoform that has only two constant domains. (It lacks the third and fourth constant domains.) This isoform has a molecular weight of about 120 kDa (5.7 S). Some birds such as ducks and geese have both full-sized and

FIGURE 37-11 The structure of lymph nodes in birds, echidna, and placental mammals.

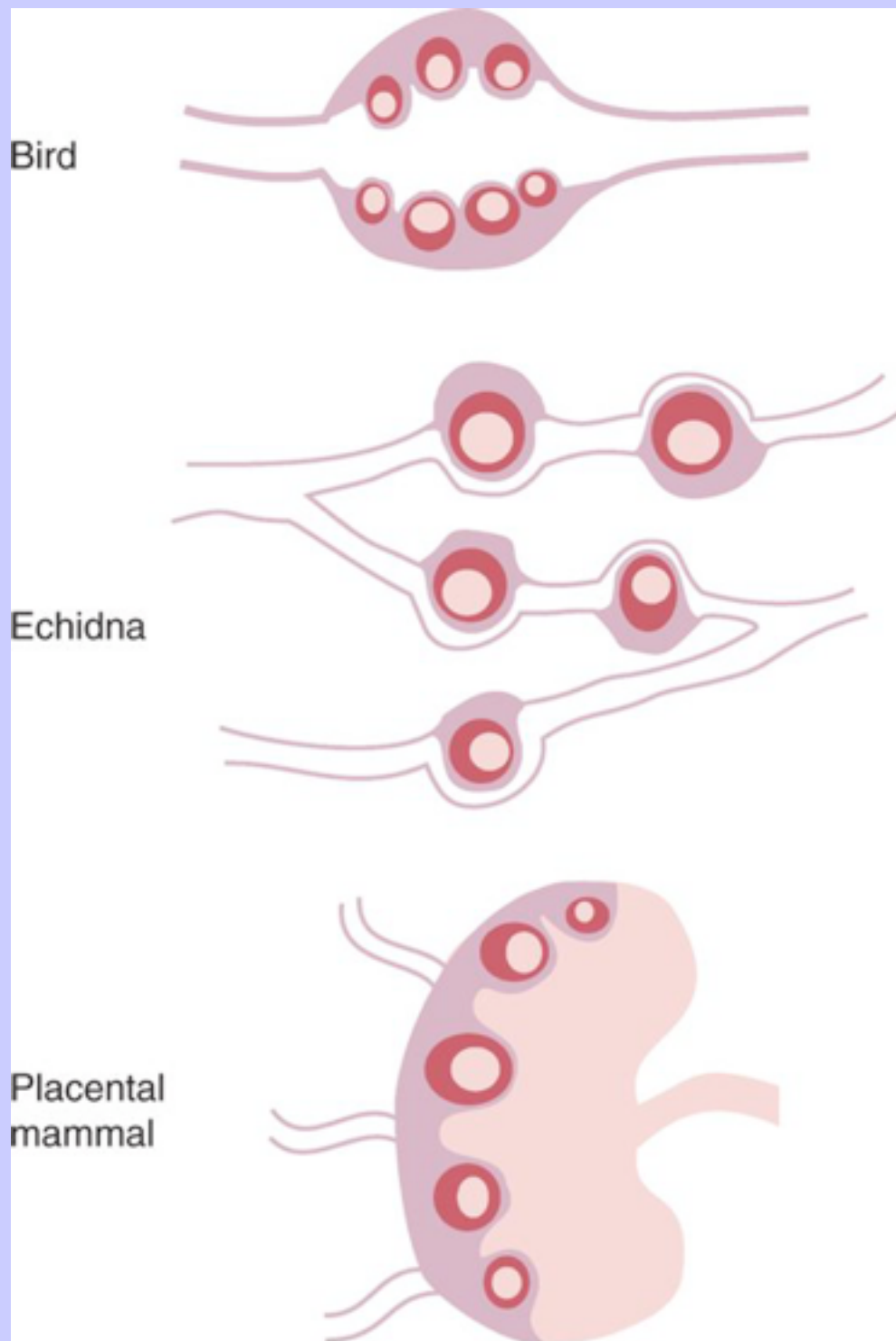
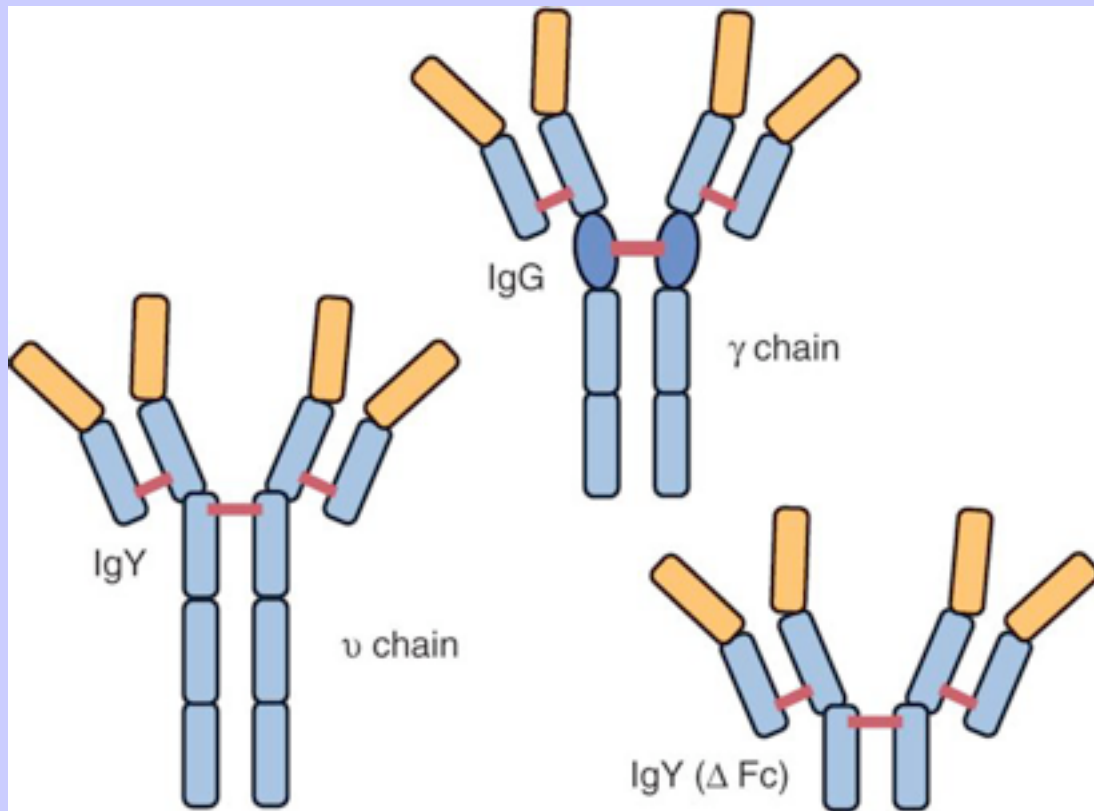


FIGURE 37-12 The structure of immunoglobulin Y (IgY) and IgY(Δ Fc) compared with mammalian IgG.



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truncated IgY. Others, such as chickens, have only full-sized molecules.

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The truncated isoform of IgY is produced as a result of alternative splicing of heavy chain mRNA. Its correct name is therefore IgY(Δ Fc). Because the molecule lacks an Fc region, it cannot activate complement or bind to Fc receptors. Its function is unclear. There has, however, been a tendency during evolution to make low-molecular weight immunoglobulins. Thus similar truncated immunoglobulins have also been described in some fish (IgM(Δ Fc)), some turtles, and the quokka (*Setonix brachyurus*), a marsupial. These low-molecular weight molecules may offer some selective advantage. For example, it has been suggested that they will not trigger potentially lethal hypersensitivity reactions. Evidence from mallards (*Anas platyrhynchos*) suggests that the ratio of IgY(Δ Fc) to intact IgY affects the efficiency of phagocytosis and determines whether immune complexes are phagocytosed in the spleen or liver.

Both isoforms of IgY lack a hinge region. Thus, although bivalent, these molecules are somewhat inflexible and can cause precipitation or agglutination only in the presence of high-salt concentrations. They tend to show somewhat restricted diversity and limited affinity maturation. Studies on the interrelationships of the vertebrate immunoglobulins clearly show that IgY is related to both IgG and IgE in mammals (see [Figure 37-9](#)). In fact, it may have arisen from an evolutionary precursor of these two classes.

It is of interest to note that chickens can develop anaphylaxis. The signs of acute anaphylaxis in chickens and other birds are similar to those in mammals, although it is likely mediated by IgY. They show increased

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salivation, defecation, ruffling of feathers, dyspnea, convulsions, cyanosis, collapse, and death. The major target organ is probably the lung, and death is due to pulmonary arterial hypotension, right-sided heart dilation, and cardiac arrest. The pharmacological agents involved include histamine, serotonin, the kinins, and leukotrienes.

37.8.2.2 Immunoglobulin M

Birds produce primary and secondary responses in a manner similar to mammals. The predominance of IgM production in the primary immune response and of IgY in the secondary response is less marked than in mammals. A monomeric IgM can be detected in chicken eggs and in 1-day-old chicks. It is thought to be derived from oviduct secretions in the hen.

37.8.2.3 Immunoglobulin A

The structure of chicken IgA is similar to IgA in mammals. The only significant difference is that chicken IgA has four heavy chain C domains, whereas mammalian IgA has only three. Chicken serum IgA exists in both dimeric (340 kDa) and monomeric (170 kDa) forms. Intestinal IgA is associated with secretory component (SC).

37.8.3 Generation of Antibody Diversity

Chickens generate antibody diversity in a manner quite unlike that seen in mammals. Chickens have only one functional V gene and one J gene for both light and heavy chains, although they do have 16 different D genes. Chicken immunoglobulin diversity is therefore generated by gene conversion. Although they have only one functional V gene, chickens have a large number of V pseudogenes that serve as sequence donors that, by gene conversion, diversify the functional light chain V gene. During recombination of the V and J genes, single bases are also added to each gene (N-region addition), and joining occurs at random. Chicken immunoglobulins are further diversified by somatic hypermutation and imprecise V-J joining.

A second major difference involves the timing of the process. In mammals, rearrangement of immunoglobulin genes is an ongoing process. Chickens, in contrast, rearrange their immunoglobulin genes as a single wave between 10 and 15 days of embryogenesis, at a period when there is clonal expansion of B cells in the bursa of Fabricius. During that 5-day period, birds generate all the antibody specificities they will need for the rest of their lives. After the bursa degenerates at puberty, the chicken must largely make do with the B cell diversity generated in early life. However, once a mature chicken B cell is stimulated by exposure to an antigen, it can generate additional V-region diversity by further gene conversion. If gene conversion is blocked in a chicken, somatic mutation can occur. Indeed, species that undertake gene conversion also show limited somatic mutation although the reverse is not true. The chicken can generate about 10^6 different immunoglobulin molecules. This is approximately one order of magnitude less than the mouse.

Chicken T cells can participate in delayed hyper-sensitivity reactions, graft-versus-host disease, and allograft rejection. Avian homologs of mammalian γ/δ TCR (TCR-1) and α/β TCR (TCR-2 and TCR-3) have been identified. TCR-2 and TCR-3 are subsets of α/β TCRs that use distinctly different V_β gene segments. TCR-2 cells undergo V-DJ joining by gene deletion, whereas TCR-3 cells undergo V-DJ joining by chromosome inversion. The structure of the avian CD3 signaling complex is different from that in mammals insofar as it contains only two dimers, $\delta/\gamma-\epsilon$ and $\zeta-\zeta$, rather than three. There is evidence that chickens possess both Th1 and Th2 cells. For example, chicken IL-18 stimulates IFN- γ release from CD4⁺ T cells.

Birds reject skin allografts in about 7 to 14 days. Histological examination shows massive infiltration of the grafted tissue with lymphocytes. These cells are believed to be T cells, since neonatal thymectomy results in a failure to reject grafts. If chicken T cells are dropped onto the chorioallantoic membrane of 13- to 14-day-old chick embryos, the cells will attack the chick tissues. This will result in pock formation on the membrane and splenic enlargement. The grafted cells attack the hematopoietic cells of the recipient. A few days after hatching, chicks become resistant to this form of graft-versus-host attack.

37.9 IMMUNITY IN MONOTREMES AND MARSUPIALS

The least-evolved mammals, the monotremes such as the duck-billed platypus (*Ornithorhynchus anatinus*) and the echidna (*Tachyglossus aculeatus*), have a spleen, thymus, and gut-associated lymphoid tissues that are as well developed as those in marsupials and eutherian mammals. However, instead of typical mammalian lymph nodes, they have lymphoid nodules that consist of several lymphoid nodules, each containing a germinal center suspended by its blood vessels within the lumen of a lymphatic plexus. Thus, each nodule is bathed in lymph. There is usually just one germinal center per nodule. The evolution of the predominant blood immunoglobulin from IgY to IgG probably occurred very early in mammalian evolution since monotremes possess not IgY but IgG. They have two IgG subclasses, IgG1 and IgG2, as well as IgE and IgM. Although distinctly different from marsupial and eutherian IgG and IgE, these show overall structural similarity to other mammalian immunoglobulins. Thus all the major structural changes that gave rise to the immunoglobulin classes expressed in modern mammals evolved before the separation of the monotremes from the marsupials and placental mammals and probably soon after the split from reptile lineages 300 Myr ago. Monotremes, like other mammals, produce predominantly IgM in the primary immune response and IgG in secondary immune responses.

The recent complete sequencing of the genome of the marsupial opossum *Monodelphis domestica* has allowed investigators to look at its immune system genes (its immunome) in detail. It contains genes for all the key immune gene families. There has been substantial duplication or gene conversion involving leukocyte receptors, NK complexes, immunoglobulins, type I interferons, and defensins. The opossum genome also contains a new TCR chain that is expressed early in development before conventional TCRs and may provide protection during the first few days of life before the opossum immune system is functional. This receptor chain, called TCRm, consists of V, D, and J genes either recombined as in eutherian mammals or prejoined in the germ-line DNA. It resembles a TCR isoform from sharks and may represent the remains of a very ancient receptor system.

Marsupials produce immunoglobulins in a manner similar to eutherian mammals. They possess four immunoglobulin isotypes: IgM, IgG, IgE, and IgA. The marsupial opossum (*Didelphis*) resembles more primitive vertebrates in that it responds well to particulate antigens such as bacteria, but responds poorly to soluble antigens. When opossums were inoculated with sheep red blood cells, the primary immune response was long lived and reasonably strong. The secondary response was weaker than the first and lasted for a much shorter period.

Mammals possess a very large number of V gene segments. When the sequences of these are analyzed, they can be shown to form different Vh gene families. Thus 7 gene families have been identified in humans and 15 in mice. Further analysis of these families shows that they form three major “clans” (clans I, II, and III). Comparative studies have shown that these three clans have probably existed for more than 400 Myr. Fish Vh sequences are most closely related to mammalian clan III. However, fish possess two additional clans not found in mammals. The monotremes and marsupials have Vh genes that also belong to clan III. Likewise, although chickens, rabbits, and pigs have relatively few Vh genes, the V genes of these three species belong to clan III. This has led to the assumption that clan III is the most ancient of the mammalian clans. However, cattle and sheep also express only a

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single Vh gene family, and this belongs to clan II. This may be due to inactivation and loss of clan III in these species.

37.10 MAMMALIAN PHYLOGENY

This book has focused on immunity in a small group of domestic mammals. These mammals have been selected not as representatives of mammalian diversity but for the behavioral traits that lend them to domestication or for the ease with which they are maintained in captivity. If we examine their place in mammalian phylogeny (Figure 37-13), we can see that most domestic animal species are relatively closely related. Even domestic pets such as dogs and cats are closer to farm animal species than to primates. Likewise, laboratory animals tend to cluster in a separate group. It is unsurprising, therefore, that significant differences exist among the immune systems of species of interest to veterinarians. It is also clear that if we are to understand the significance of these differences and how they evolved, we must examine the immune

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FIGURE 37-13 The currently accepted phylogeny of mammals as based upon analysis of gene sequences. Note that none of the domestic animal species can be considered representative of mammals as a whole.

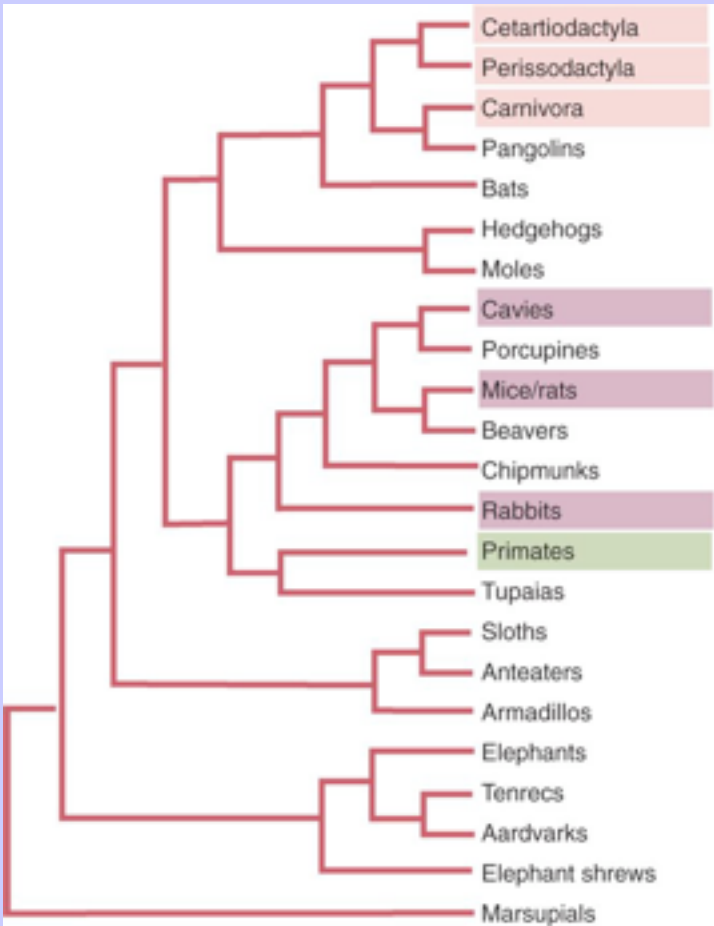
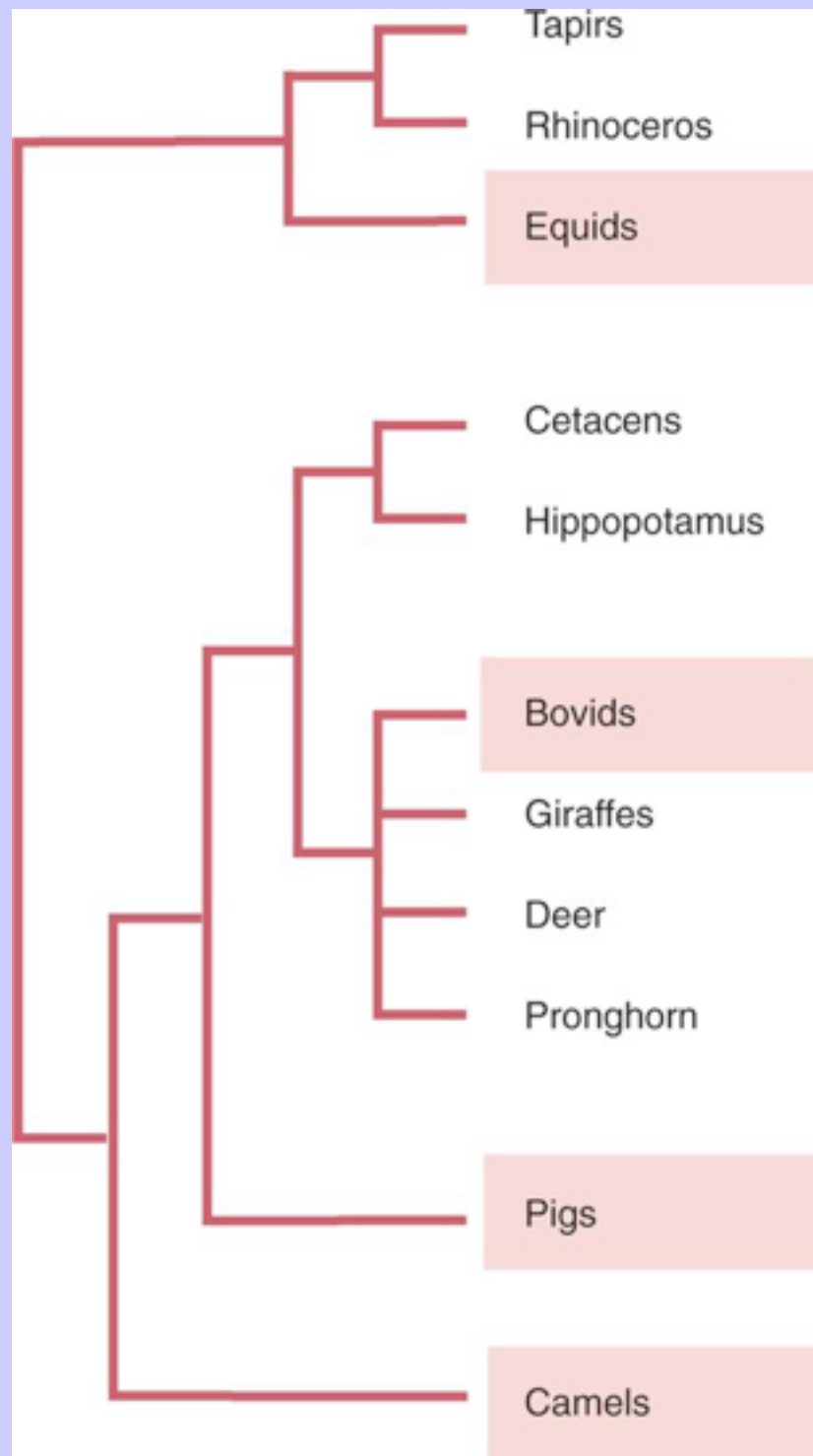


FIGURE 37-14 The molecular phylogeny of the domestic herbivores. Many gaps remain in our knowledge of the immunology of these species.



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systems of other, unrelated mammals. Even within the major domestic herbivores ([Figure 37-14](#)), their phylogeny demonstrates why there are significant differences between their immune systems.

37.1 FEVER

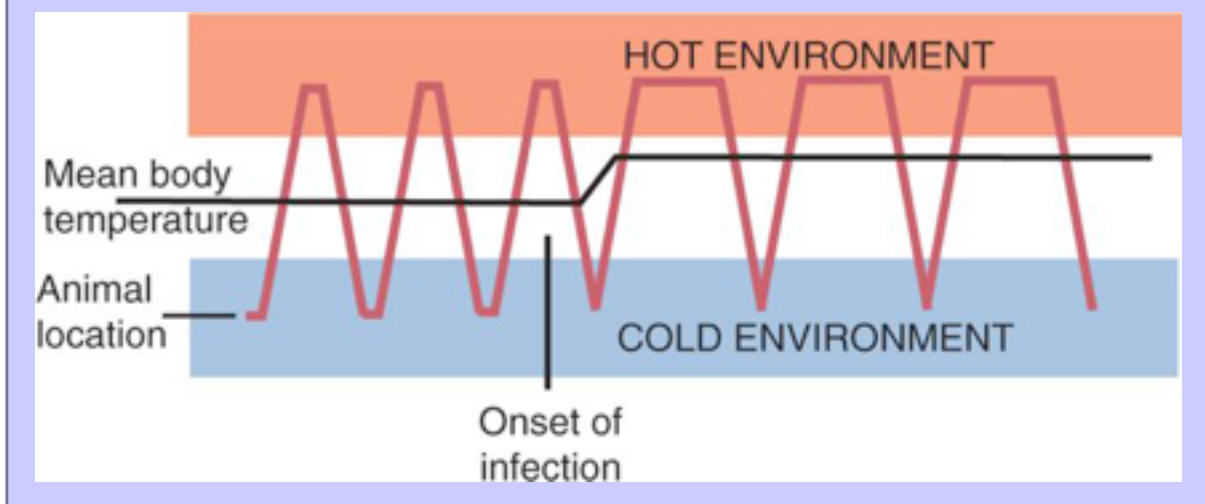
Vertebrates generally respond to antigens faster and more intensely at higher temperatures. Conversely, low temperatures in ectotherms may be significantly immunosuppressive. Thus in chilled fish, the lag period following vaccination may be long or there may be a complete absence of a detectable antibody response. Only certain phases of the antibody response are temperature dependent. For example, secondary immune responses can be elicited at low temperatures provided primary immunization is carried out at a high temperature. The cells that are sensitive to low temperatures in fish are helper T cells, and the effect is due to a loss of T cell membrane fluidity and reactivity to interleukins. Acclimatization to low temperatures can also occur. For example, goldfish that are acclimatized at a low temperature may be able to produce a quantity of antibody-forming cells similar to the quantity produced by those that have remained at a warmer temperature. The nature of the antigen is also critical in that certain T cell-dependent mitogens are ineffective at low temperatures, again implying that the target cell is a helper T cell. The environmental temperature influences the rejection of allografts in all ectotherms.

Although it is well recognized that most endotherms such as mammals develop a fever when infected, it is less apparent that ectotherms such as fish or reptiles and even arthropods also develop fever in response to infection. Ectotherms are unable to change their body temperature by physiological mechanisms. As a result, they cannot develop a fever if maintained in a constant temperature environment. If, however, they are maintained in an environment with cool and warm areas, they will cycle between these areas and maintain their body temperature within well-defined limits. For example, it has been observed that normal iguanas (*Dipsosaurus dorsalis*) maintain their temperature between 37° and 41° C. However, iguanas infected with the bacterium *Aeromonas hydrophila* modify their behavior so that they spend more time in the warm environment ([Figure 37-15](#)). As a result, their temperatures cycle between 40° and 43° C. Once the bacterial

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FIGURE 37-15 A fever can be induced in ectotherms by modifications in behavior. Simply spending more time in a warm environment will effectively raise average body temperature. This behavioral response occurs in response to microbial infection.



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infection is cured, the iguanas resume their normal behavior. Thus the iguanas effectively induce a fever by their behavior. A similar behavioral fever is seen in goldfish maintained in two interconnected tanks maintained at different temperatures. In response to microbial infection, the fish will choose to spend more time in the warmer water, thus effectively raising their body temperature. The benefits of this to ectotherms are obvious, because, as pointed out above, their immune systems function much more efficiently at higher temperatures. Many insects also respond to fungal or bacterial infections by developing a behavioral fever. That is, they raise their mean body temperature by spending more time in a warmer environment. It is interesting to note, however, that not all insect pathogens can stimulate such a response and not all insects respond in the same way. For example, the bacterium *Serratia marcescens* can elicit a fever in the desert locust but not in the domestic cricket.

Some mammals hibernate, most notably bears, bats, and some rodents. At this time, their body temperature may fall. If bats are cooled to around 8° C, they cease antibody production, but rewarming permits rapid resumption of antibody synthesis. This cessation of the antibody response in hibernating bats may allow them to act as persistent carriers of viruses such as rabies.

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³⁸ CHAPTER 38 Immunodiagnostic Techniques

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38.1 KEY POINTS

- Tests for serum antibodies may be used to detect the presence of an infectious disease. Specific antibodies may also be used to identify an unknown antigen.
- The most sensitive and specific tests directly detect the antigen or antibody of interest. These are called primary binding tests. An example of a primary binding test is an enzyme-linked immunosorbent assay.
- Secondary binding tests tend to be the easiest to perform but are less sensitive than primary binding tests. Examples include precipitation and agglutination tests.
- Tertiary tests directly measure protection. They are usually complex and so may not lend themselves to rapid testing. An example is a virus neutralization test.
- Serological tests are judged by the number of false positive results they generate (their specificity) and by the number of false negative tests they generate (their sensitivity).
- In general, highly sensitive tests tend to have low specificity and vice versa.

Immune responses are used in two ways to diagnose disease. First, specific antibodies may be used to detect or identify an antigen of interest. These antigens can be associated with an infectious agent or simply be molecules that need to be located or measured. Second, by detecting specific antibodies in serum, it is possible to determine whether an animal has been previously exposed to an infectious agent. This may establish a diagnosis or determine the degree of exposure of the population to that agent. The measurement of antigen-antibody interactions for diagnostic purposes is called serology.

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Table 38-1 Smallest Amount of Antibody Protein Detectable by Selected Immunological Tests

Tests	Protein (µg/ml)
Primary Binding Tests	
ELISA	0.0005
Competitive radioimmunoassay	0.00005
Secondary Binding Tests	
Gel precipitation	30
Ring precipitation	18
Bacterial agglutination	0.05
Passive hemagglutination	0.01
Hemagglutination inhibition	0.005
Complement fixation	0.05
Virus neutralization	0.00005
Bactericidal activity	0.00005
Antitoxin neutralization	0.06
In Vivo Test	
Passive cutaneous anaphylaxis	0.02

Serological techniques can be classified into three broad categories. The first consists of primary binding tests, which directly measure the binding of antigen to antibody ([Table 38-1](#)). Secondary binding tests, which measure the results of antigen-antibody interaction in vitro, comprise the next category. These tests are usually less sensitive than the primary binding tests but may be simpler to perform or require simpler technology. In vivo tests, the third category, measure the actual protective effect of antibodies in an animal.

38.2 REAGENTS USED IN SEROLOGICAL TESTS

38.2.1 Serum

The most common source of antibodies is serum obtained from clotted blood. Serum may be stored frozen and tested when convenient. If necessary the serum can be depleted of complement activity by heating to 56° C for 30 minutes.

38.2.2 Antiglobulins

Because immunoglobulins are complex proteins, they are antigenic when injected into an animal of a different species. For example, purified dog immunoglobulins can be injected into rabbits. The rabbits respond by making specific antibodies called antiglobulins. Depending on the purity of the injected immunoglobulin, it is possible to

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make nonspecific antiglobulins against immunoglobulins of all classes or very specific antiglobulins directed against single classes. Antiglobulins are essential reagents in many immunological tests. They are commercially available, and one antiglobulin can be used to detect all immunoglobulins from the same species.

38.2.3 Monoclonal Antibodies

Hybridoma-derived monoclonal antibodies are pure and specific, can be used as standard chemical reagents, and can be obtained in almost unlimited amounts (see [Chapter 13](#)). As a result, monoclonal antibodies frequently replace conventional antiserum as reagents in immunodiagnostic tests.

38.3 PRIMARY BINDING TESTS

Primary binding tests are performed by allowing antigen and antibody to combine and then measuring the immune complexes formed. In order to measure these reactions, one of the reactants must be chemically labeled. Radioisotopes, fluorescent dyes, colloidal metals, and enzymes have all been used as labels in these tests.

38.4 RADIOIMMUNOASSAYS

Assays that use radioisotopes as labels have the advantage of being exquisitely sensitive. On the other hand, isotope detection systems are expensive. This expense, combined with the hazards of radioactivity and the need to safely dispose of radioactive material, makes radioimmunoassays a practical choice only when highly sensitive assays are required.

38.4.1 Radioimmunoassays for Antibody

The radioallergosorbent test measures specific immunoglobulin E (IgE) in the serum of allergic animals. In this technique, antigen-impregnated cellulose disks are immersed in test serum so that any antibody binds to the antigen. After washing to remove unbound antibody, the disk is immersed in a solution containing radiolabeled antiglobulin (e.g., anti-IgE). The antiglobulin binds only if IgE has bound to the antigen. The amount of radioactivity bound to the disk is therefore a measure of the level of specific IgE antibody activity in the serum.

38.4.2 Radioimmunoassays for Antigen

Competitive immunoassays are based on the principle that unlabeled antigen will displace radiolabeled

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FIGURE 38-1 The principle of competitive radioimmunoassay. Unlabeled antigen in the test solution displaces labeled antigen from immune complexes. The amount of labeled antigen released will be proportional to the amount of unlabeled antigen added.

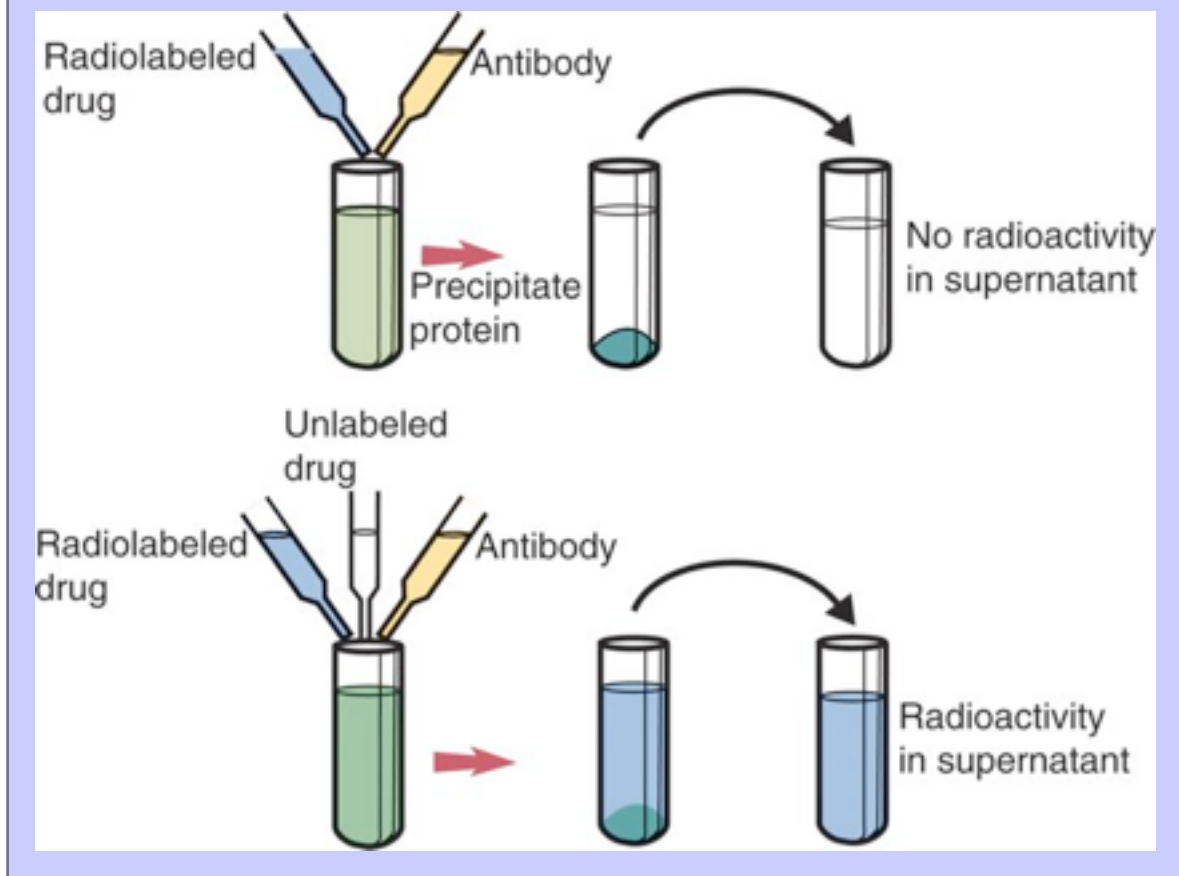
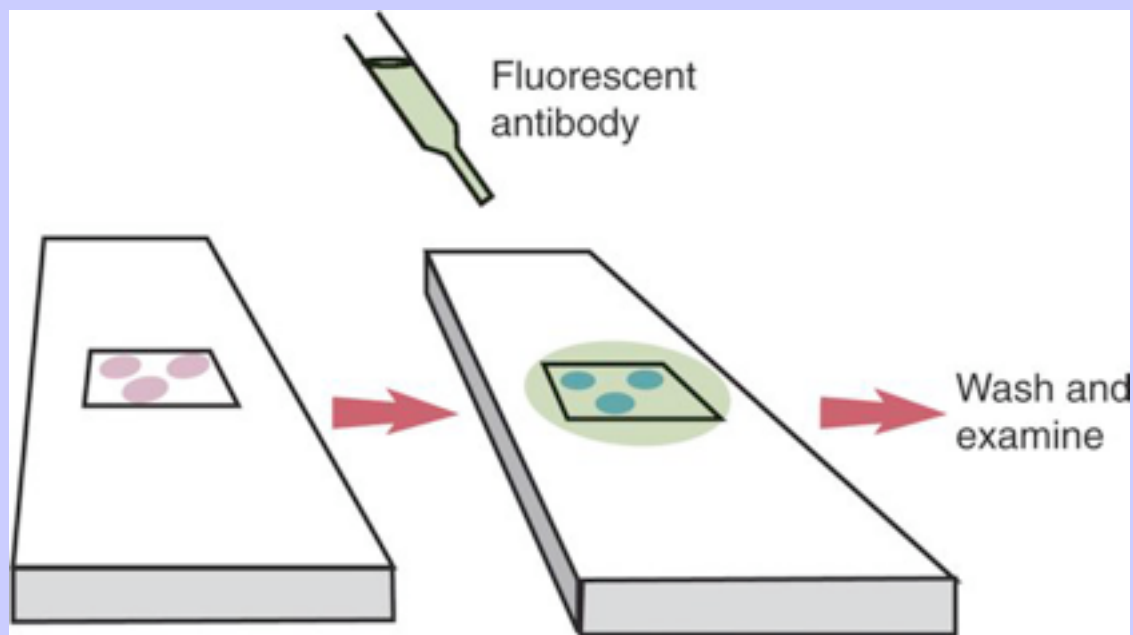


FIGURE 38-2 The direct fluorescent antibody assay. This technique is used to detect antigen by means of fluorescein isothiocyanate–labeled antibody.



antigen from immune complexes ([Figure 38-1](#)). These tests are exquisitely sensitive and so are commonly used to detect trace amounts of drugs. The antigen (or drug) is labeled with a radioactive isotope such as tritium (H^3), carbon 14, or iodine 125. When radio-labeled antigen is mixed with its specific antibody, they combine to form immune complexes that can be precipitated out of solution. Any radioactivity remaining in the supernatant fluid is due to the presence of unbound antigen. If unlabeled antigen is added to the mixture before adding the antibody, it will compete with the radioactive antigen for antibody-binding sites. As a result, some labeled antigen will be unable to bind, and the amount of radioactivity in the supernatant will increase. If a standard curve is first constructed based on the use of known amounts of unlabeled antigen, then the amount of antigen in a test sample may be measured by reference to this standard curve.

38.5 IMMUNOFLUORESCENCE ASSAYS

Fluorescent dyes are commonly employed as labels in primary binding tests, the most important being fluorescein isothiocyanate (FITC). FITC is a yellow compound that can be chemically linked to anti-bodies without affecting their reactivity. When radiated with invisible ultraviolet or blue light at 290 and 145 nm, FITC re-emits visible green light at 525 nm. This fluorescence can be readily seen using a fluorescent microscope. FITC-labeled antibodies are used in the direct and indirect fluorescent antibody tests.

38.5.1 Direct Fluorescent Antibody Tests

Direct fluorescent antibody tests are used to identify the presence of antigen in a tissue sample. Antibody directed against a specific antigen such as a bacterium or virus is first labeled with FITC. A tissue section or

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smear containing the organism is fixed to a glass slide, incubated with the labeled antiserum, and then washed to remove any unbound antibody ([Figure 38-2](#)). When examined by darkfield illumination under a microscope with an ultraviolet light source, the organisms that bind the labeled antibody will fluoresce brightly. This test can identify the presence of small numbers of bacteria in a sample. For example, it can be used to detect *M. avium ssp. paratuberculosis* in feces, or to detect bacteria such as *Dichelobacter nodosus*, *Listeria monocytogenes*, or the clostridial organisms in diseased tissues ([Figure 38-3](#)). It may also be employed to detect viruses in tissue culture or in tissues from infected animals. Examples include the detection of rabies virus in the brains of infected animals or feline leukemia virus in infected leukocytes (see [Chapter 35](#), [Figure 35-3](#)).

38.5.2

Indirect Fluorescent Antibody Tests

Indirect fluorescent antibody tests can be used to measure antibodies in serum or to identify specific antigens in tissues or cell cultures. When antibody levels are measured, antigen is employed as a tissue smear, section, or cell culture on a slide or coverslip. This is incubated in serum suspected of containing antibodies to that antigen. The serum is then washed off, leaving only specific antibodies bound to the antigen ([Figure 38-4](#)). These bound antibodies may then be visualized by incubating the smear in FITC-labeled antiglobulin. When the unbound labeled antiglobulin is removed by washing and the slide examined, the presence of fluorescence indicates that antibody was present in the test serum. The quantity of

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FIGURE 38-3 Direct immunofluorescence of a smear of *Clostridium septicum* (see also [Chapter 19](#), [Figure 19-7](#); and [Chapter 35](#), [Figure 35-3](#)). (Courtesy Dr. John Huff.)



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antibody in the test serum may be estimated by examining increasing dilutions of serum on different antigen preparations.

The indirect fluorescent antibody test has two advantages over the direct technique. Since several labeled antiglobulin molecules will bind to each antibody molecule, the fluorescence will be considerably brighter than in the direct test. Similarly, because the antiglobulins used are specific for each immunoglobulin class, the class of the specific antibody may also be determined.

38.5.3

Particle Concentration Fluorescence Immunoassays

Immunofluorescence assays can be automated and quantitated by means of particle immunoassays ([Figure 38-5](#)). For example, antigen-coated, submicro-meter polystyrene particles can be mixed with test

FIGURE 38-4 The indirect fluorescent antibody test may be used to detect either antigen or antibody. In a section smear or culture, the antigen will bind antibody from serum. After washing, this antibody may be detected by binding to fluorescein isothiocyanate-labeled antiglobulin.

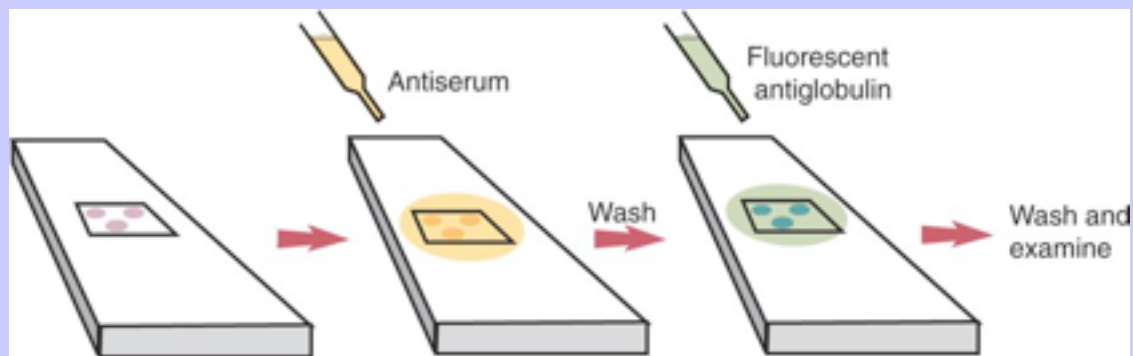


FIGURE 38-5 The principle of the particle concentration fluorescence immunoassay.

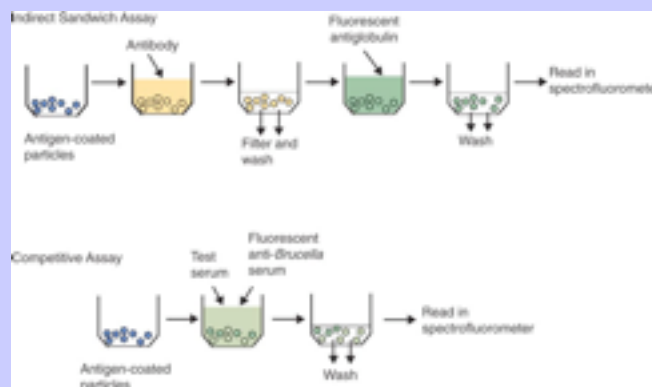
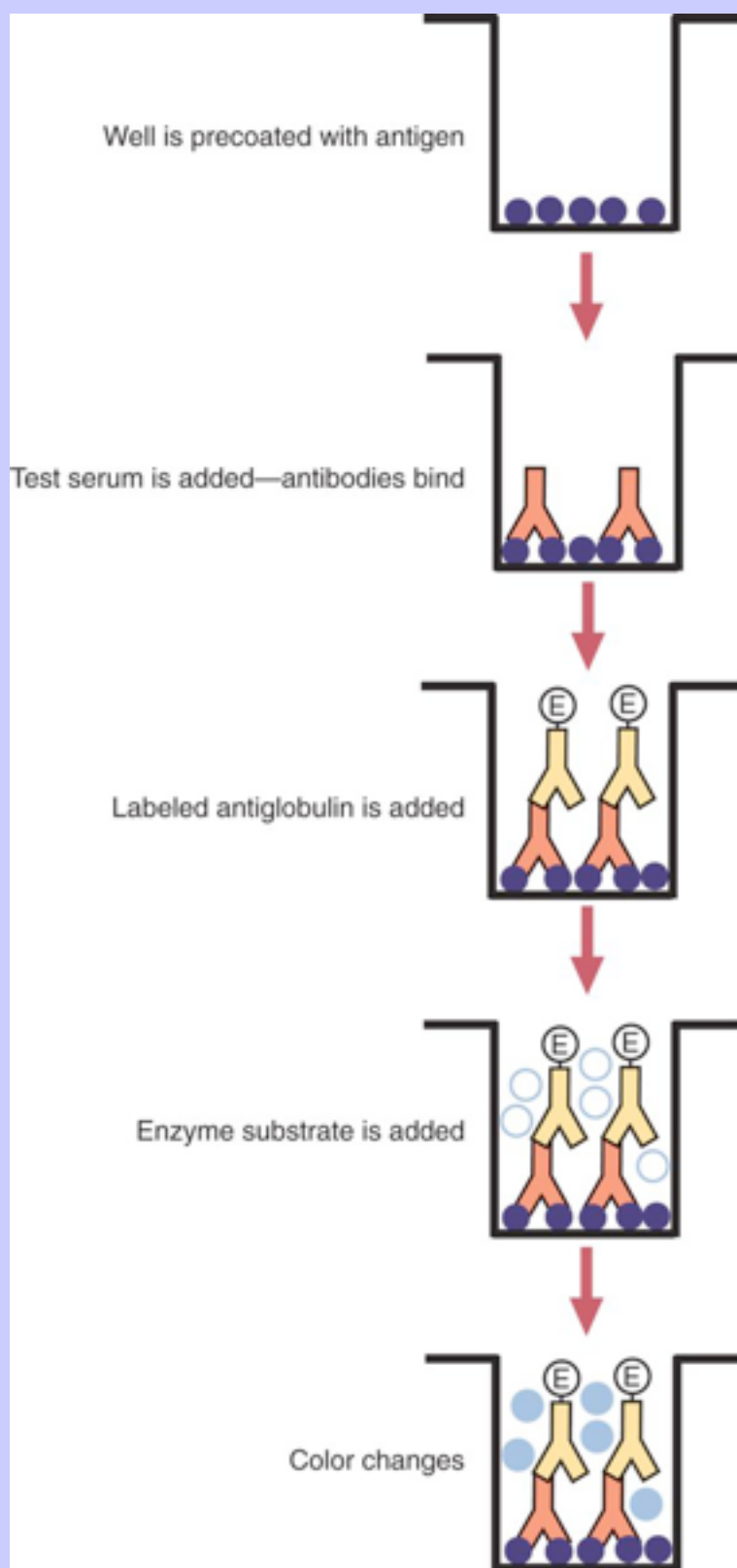


FIGURE 38-6 The indirect enzyme-linked immunosorbent assay (ELISA) technique. Antigen is bound to the wells in a styrene plate. The presence of bound antibody is detected by means of an enzyme-labeled antiglobulin. Addition of the enzyme substrate leads to a color change proportional to the amount of bound antibody. This color change can be estimated visually or read in an ELISA reader (a specially adapted spectrophotometer).



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serum. After incubation, the particles are recovered by vacuum filtration, washed to remove unbound antibody, and exposed to a fluorescent antiglobulin. After filtering the suspension again and washing to remove unbound antiglobulin, the particle suspension can be placed in a spectrofluorometer and the intensity of particle-bound fluorescence measured. This provides a measure of the level of antibodies in the test serum. A very useful variation on this is the competitive assay used as a rapid test for antibodies to *Brucella abortus* in cattle. In this case, *Brucella antigen*-coated poly-styrene particles are mixed with a standard amount of fluorescent anti-*Brucella* serum and the serum under test. If positive, the unlabeled test serum inhibits the binding of fluorescent antibodies to the particles. The more antibody in the test serum, the greater the inhibition of fluorescent antibody binding.

38.6 IMMUNOENZYME ASSAYS

Among the most important immunoassays employed in veterinary medicine is the enzyme-linked immunosorbent assay (ELISA). As with other primary binding tests, ELISAs may be used to detect and measure either antibody or antigen.

38.6.1 Microwell ELISA Tests

The most common form of ELISA is used to detect and measure specific antibodies. In order to perform this assay, microwells in polystyrene plates are first filled with an antigen solution ([Figure 38-6](#)). Because proteins bind firmly to polystyrene surfaces, the wells remain coated with a layer of antigen after unbound antigen is removed by vigorous washing. These coated plates can be stored until required. When testing serum, the serum is added to the wells. Any antibodies in the serum will bind to the antigen layer. After incubation and washing to remove unbound antibody, the presence of any bound antibodies is detected by adding a solution containing an antiglobulin chemically linked to an enzyme. This labeled antiglobulin binds to the antibody and, following incubation and washing, can be detected and measured by adding a solution containing the enzyme substrate. The enzyme and substrate have been selected to ensure that a colored product develops in the tube. The intensity of the color that develops is therefore proportional to the amount of enzyme-linked antiglobulin that is bound, which in turn is proportional to the amount of antibody present in the serum under test. The color intensity may be estimated visually or, preferably, by spectrophotometry.

One modification of this technique is the antibody sandwich ELISA, which can be used to detect and measure a specific antigen ([Figure 38-7](#)). The wells in polystyrene plates are coated with specific antibody (capture antibody) before testing. To conduct the test, the antigen solution to be tested is added to each well. The capture antibody will bind any antigen present in the test solution. This step is followed, after washing, by specific antibody, which also binds the antigen (the detection antibody). The plates are again washed to remove unbound antibody. Next enzyme-labeled antiglobulin and substrate are added, in the same manner

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FIGURE 38-7 The antibody sandwich enzyme-linked immunosorbent assay. Antigen is bound to the plate by means of an antibody. The presence of that bound antigen is detected by sequential addition of a second antibody and an enzyme-labeled antiglobulin. Addition of the enzyme substrate leads to a color change proportional to the amount of bound antigen.

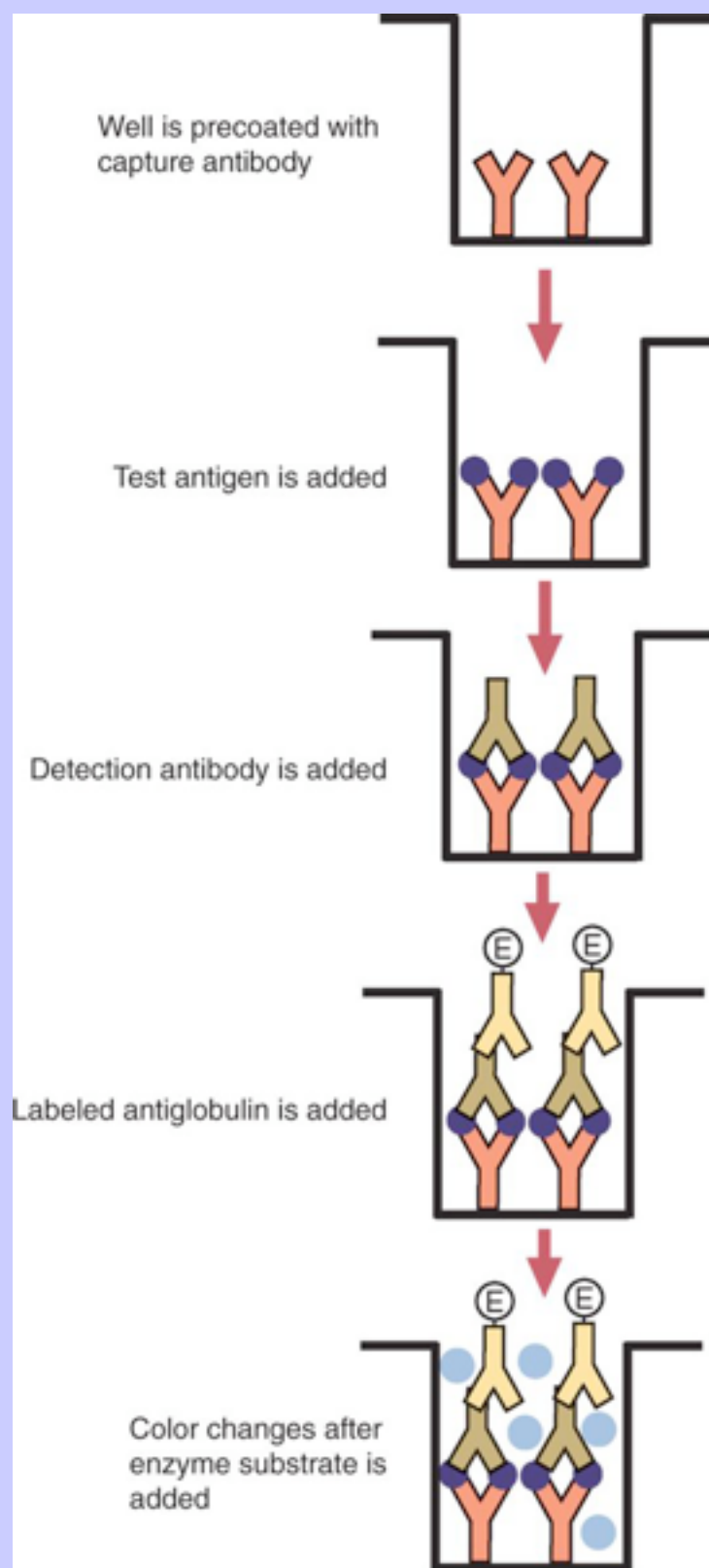
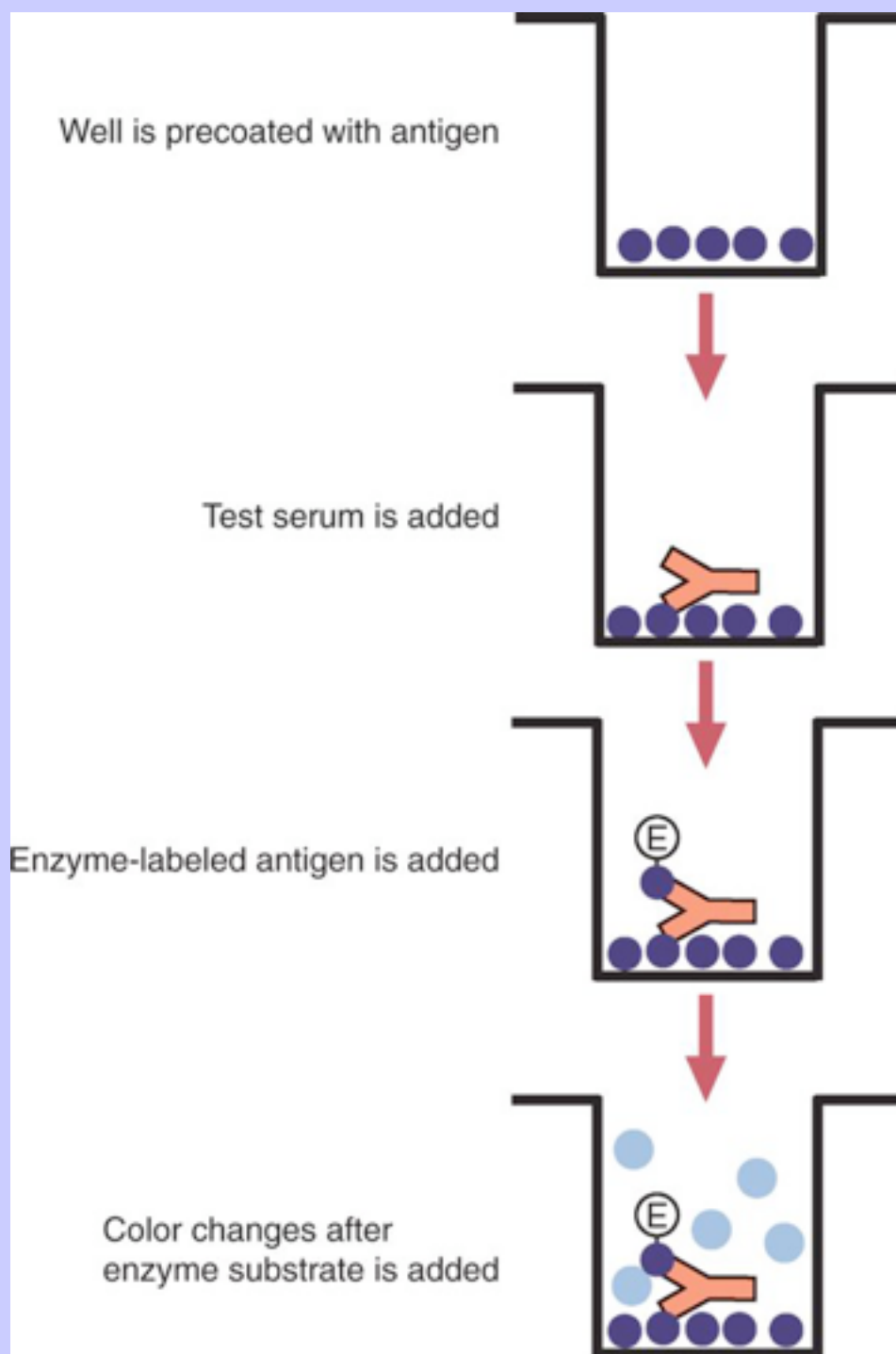


FIGURE 38-8 The labeled antigen enzyme-linked immunosorbent assay. The serum under test is added to an antigen-coated plate. Bound antibodies are then detected by an enzyme-labeled antigen.



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as for the indirect technique described earlier. (It is important that the capture antibody and the detection antibody be from the different species and that a species-specific antiglobulin be used for visualization of the detection antibody. This will avoid false-positive results caused by binding of the antiglobulin to the capture antibody in the absence of antigen.) In this assay, the intensity of the color reaction is related directly to the amount of bound antigen. Because these tests involve the formation of antibody-antigen-antibody layers, they are called sandwich ELISAs. Sandwich ELISAs are used to detect circulating virus in blood from cats with feline leukemia.

Another common modification of this technique is the labeled-antigen ELISA used to detect antibodies. This is favored in manufactured diagnostic kits. The antigen is bound to the microwells before testing ([Figure 38-8](#)). The serum to be tested is added, it is incubated and the plate is washed, and a labeled antigen is then added. Any antibody present will bind the labeled antigen to the microwell, where it can be measured. This technique works well for testing whole blood because not all the unbound antibody has to be washed out of the wells before adding the labeled antigen.

A competitive ELISA can be used to measure hapten molecules or viral antigens ([Figure 38-9](#)). In this technique, the microwell is coated with specific antibody before testing. In a single reaction, the test sample and enzyme-labeled antigen are placed in the well, where the antigens compete for the antibody-binding sites. The amount of labeled antigen bound to the microwell is inversely related to the concentration of antigen in the test sample. This technique is faster than other ELISA techniques. It can be made very sensitive if the sample antigen is permitted to react with the antibody before the labeled antigen is added.

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FIGURE 38-9 The competitive enzyme-linked immunosorbent assay. Labeled and unlabeled antigen compete for binding to antibody. Addition of the enzyme substrate leads to a color change inversely proportional to the amount of test antigen bound.

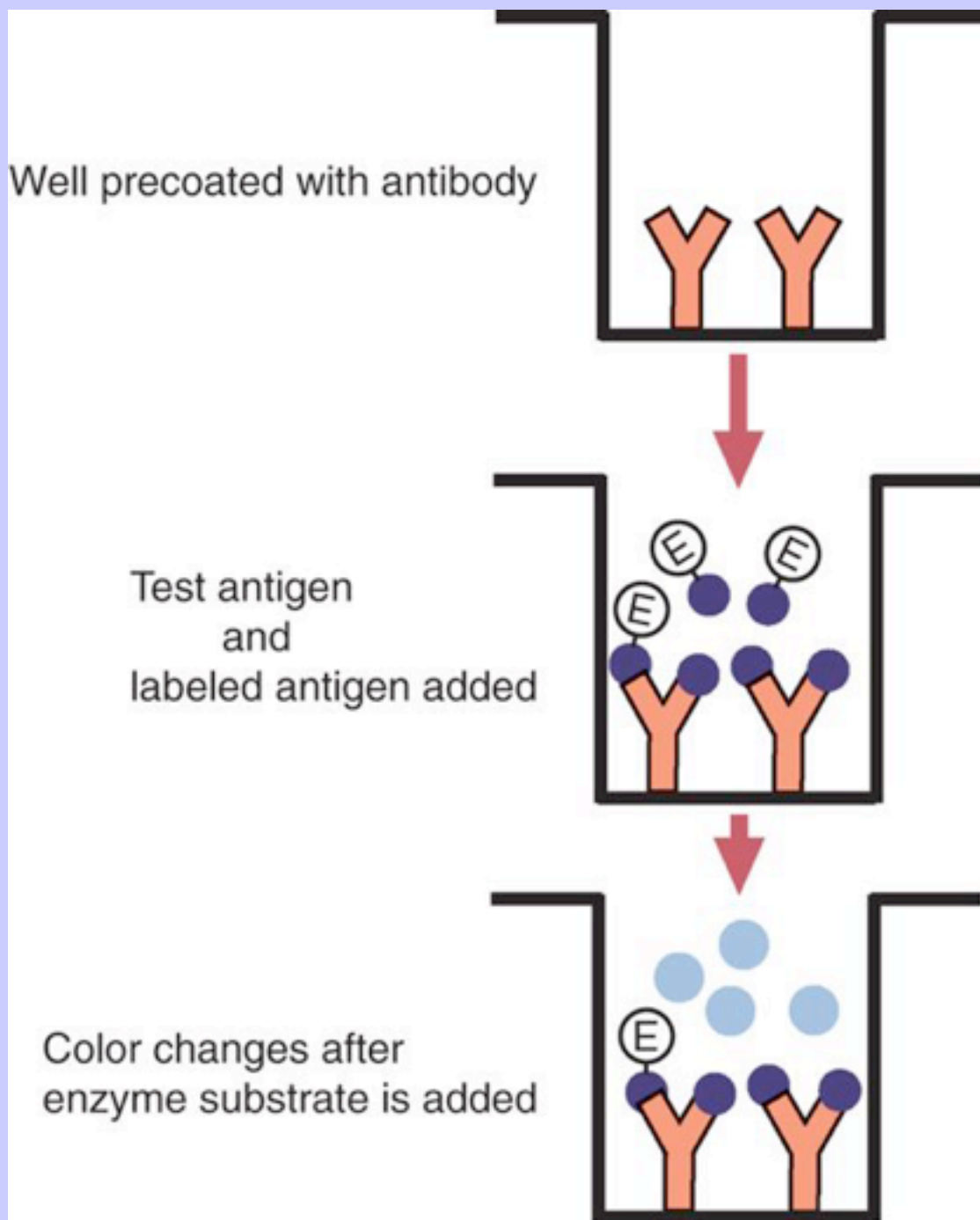


FIGURE 38-10 The Western blotting technique. Serum is separated by electrophoresis and blotted onto nitrocellulose paper; the antigen bands are revealed by use of specific antibody and an enzyme- or isotope-labeled antiglobulin. The blotting stage may be a passive transfer, or an electric potential may be used to accelerate the blotting process.

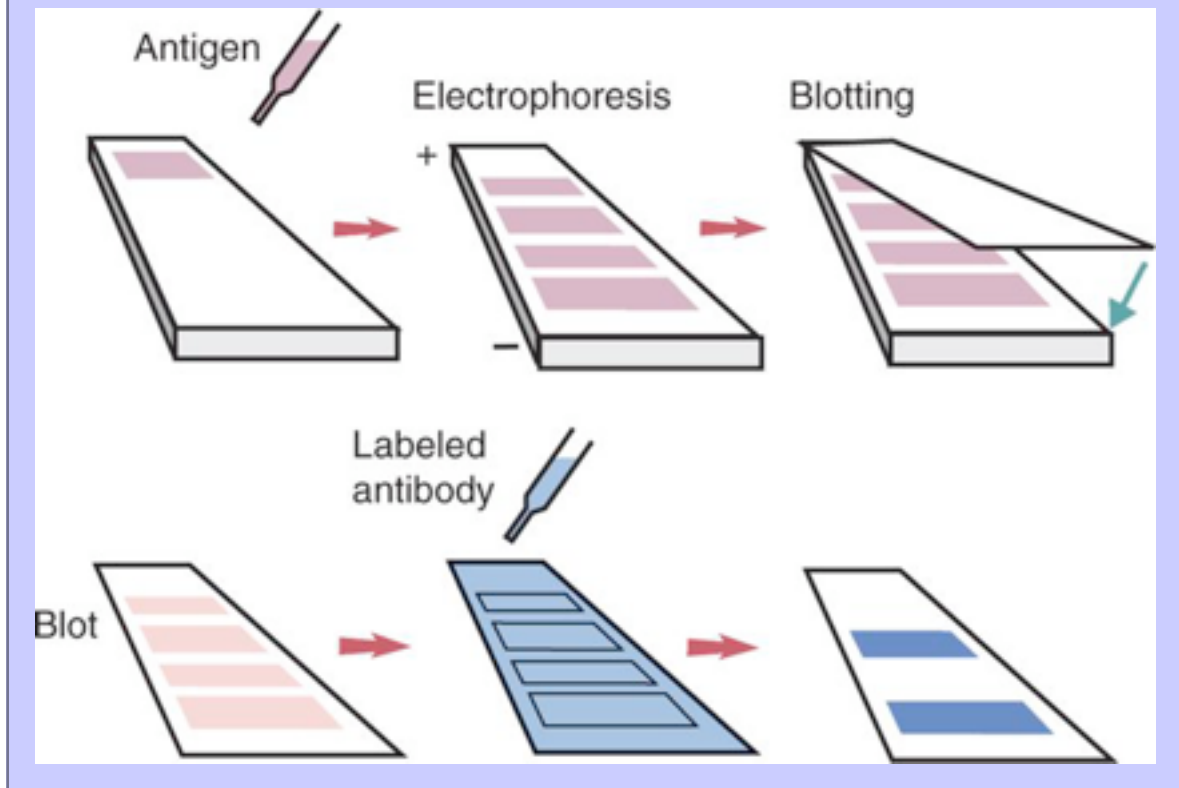
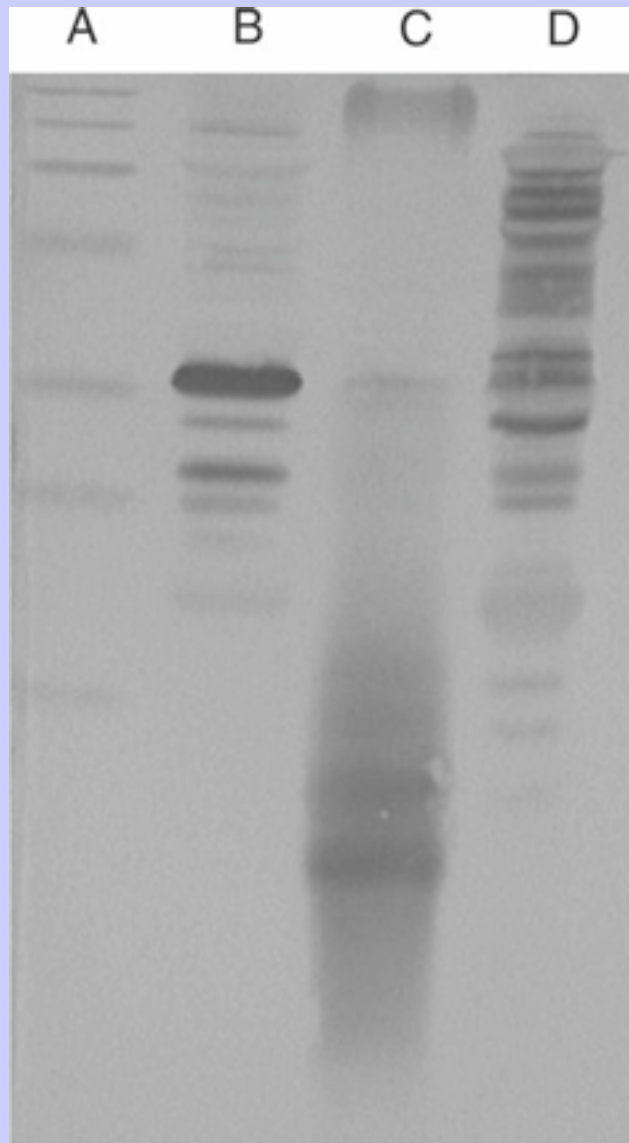


FIGURE 38-11 The allergic response to soy proteins. In this Western blot, soy extracts were first electrophoresed and blotted. The blot was then exposed to serum from a dog that was highly allergic to soy proteins. The presence of bound immunoglobulin E (IgE) was detected by exposure to labeled anti-canine IgE. Each band represents an antigen from soy recognized by the dog's IgE. **A**, Prestained molecular weight markers. **B**, Globulin fraction of whole soy. **C**, Hydrolyzed soy globulin. **D**, Whey fraction of whole soy. (Courtesy Dr. Robert Kennis.)



38.6.2 Western Blotting

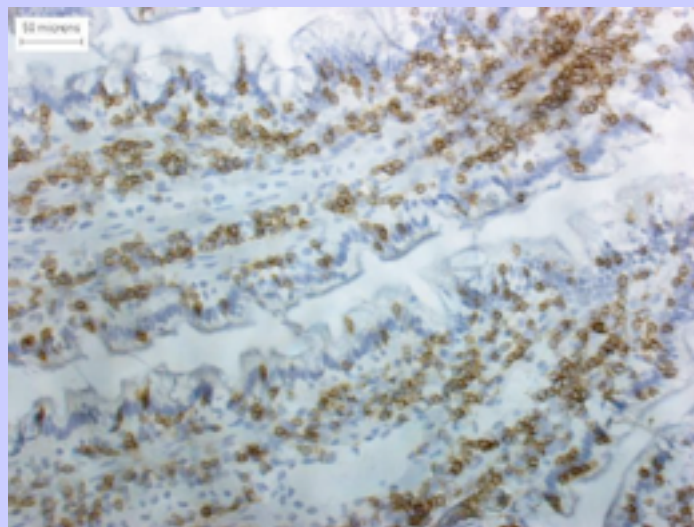
One solution to the problem of identifying protein antigens in a complex mixture is the use of a technique called Western blotting. This is a three-stage primary binding test ([Figure 38-10](#)). Stage 1 involves electrophoresis of a protein mixture on gels so that each component is resolved into a single band. Stage 2 involves blotting or transfer of these protein bands to an immobilizing nitrocellulose membrane. This is accomplished by placing the membrane on top of the gel and sandwiching the two between sponges saturated with buffer. The membrane-gel sandwich is supported between rigid plastic sheets and placed in a buffer reservoir. An electrical current is then passed between the sponges. The protein bands are transferred from the gel to the membrane without loss of resolution.

In the third stage an enzyme immunoassay or radioimmunoassay is used to visualize transferred antigens. When an enzyme immunoassay is employed the membrane is first incubated in specific antiserum. After the membrane has been washed, an enzyme-labeled antiglobulin solution is added. When this is removed by washing, substrate is added and a color develops in the bands where the antibody has bound to antigen. When isotope-labeled antiglobulin is used, an auto-radiograph must be made and the labeled band identified by darkening of a photographic emulsion. Western blotting is used to identify the important antigens in complex microorganisms or parasites ([Figure 38-11](#)). A variant form of the Western blot is the dot blot. Antigen solution is drawn through a nitrocellulose membrane so that any protein binds to the membrane. The presence of the antigen can be determined using specific antiserum and enzyme-labeled antiglobulin in sequence. After exposure to enzyme substrate, the

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FIGURE 38-12 The immunoperoxidase technique showing the presence of α/β T cells in the lamina propria and epithelium of canine duodenum. Cells binding the monoclonal antibody are exposed to peroxidase-labeled specific antiglobulin. The presence of the peroxidase is revealed as a brown deposit. (From German AJ, Hall EJ, Moore PF, et al: *J Comp Pathol* 121:249-263, 1999.)



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presence of a stained dot indicates a positive reaction. (Use of nasal washings as a source of the antigen, such as when trying to detect respiratory viruses, is called a “snot blot.”)

It is possible to put “dots” of many different monoclonal antibodies on a single sheet of nitrocellulose. They may then be exposed to a complex labeled antigen mixture such as a cell protein extract, and after washing and developing, the relative concentrations of many different antigens can be visualized. This is known as an antibody microarray.

ELISAs can be used to test fluids other than blood. For example, saliva or tears can be tested for the presence of feline leukemia virus. In most cases these are simply modified versions of the serum ELISA tests. However, in one such test, a hard plastic swab with antibody to feline leukemia virus bound to the tip is rubbed throughout the cat's mouth. The anti-bodies on the swab are protected by a sugar coating that is removed by soaking before the test. The antibody on the swab will bind any viral antigen in the saliva. The swab is then inserted into a tube containing enzyme-labeled monoclonal antibodies against feline leukemia virus antigens. After washing, the swab is placed in a solution of the enzyme substrate and the color change noted. This technique is much less sensitive than testing blood directly but is very convenient.

38.6.3 Immunohistochemistry

Enzymes conjugated to immunoglobulins or antiglobulins can be used to locate specific antigens in tissue sections. Horseradish peroxidase is the most widely employed label. The tests are performed in a manner similar to the immunofluorescence tests. Thus in the direct immunoperoxidase test, the tissue section is treated with the enzyme-labeled antibody. After washing, the tissue is incubated in a solution of the appropriate enzyme substrate. Bound antibody is detected by the development of a brown stain at the site of antibody binding ([Figure 38-12](#)). In the indirect test, bound antibody is detected by means of a labeled antiglobulin. This technique has a significant advantage over immunofluorescence techniques in that the tissue can be examined by conventional light microscopy and can be stained so that structural relationships are easier to see.

38.7 DISPOSABLE IMMUNOASSAY DEVICES

Recent years have seen the development of simple immunoassays that can be employed in the clinic and will give useful information in a very short time. These assays simply provide all necessary reagents in excess and the sample to be tested becomes the limiting feature. Most disposable devices use this form of assay because the use of excess reagents makes the accurate metering of the sample unnecessary. Examples include immunofiltration and immunochromatography assays.

38.7.1 Immunofiltration

Membrane filter or flow-through devices use a capture antibody immobilized on a membrane filter ([Figure 38-13](#)). One simple method uses a nylon membrane coated with antibody. It is set on a support base connected to an absorbent bed. A test sample, such as blood that contains antigen, flows through the antibody-coated membrane, followed at specific intervals by defined volumes of labeled antibody conjugate, wash solution, and enzyme substrate. A positive result, where antigen has bound, may be visualized as a colored dot or the creation of a plus sign. In this test, the negative sign area is formed by material that binds the enzyme conjugate (or by enzyme coupled to the matrix); the other vertical bar, where the plus sign appears, is formed by capture antibody bound to the matrix. A modification, which allows the use of whole blood, includes either a blood-solubilizing dilution system or a prefilter to remove cells. Because of the high surface area within these membranes, assay

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times can be relatively short. This form of test is commonly employed for the diagnosis of feline leukemia, feline immunodeficiency virus, and heartworm infections.

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FIGURE 38-13 An immunofiltration technique. Captured antibody is immobilized on a membrane, and reagent samples are allowed to flow through sequentially. The final labeled product is seen as a colored bar or dot. In practice, this method is used in the form of a plastic-mounted kit. (Courtesy Idexx, Inc.)

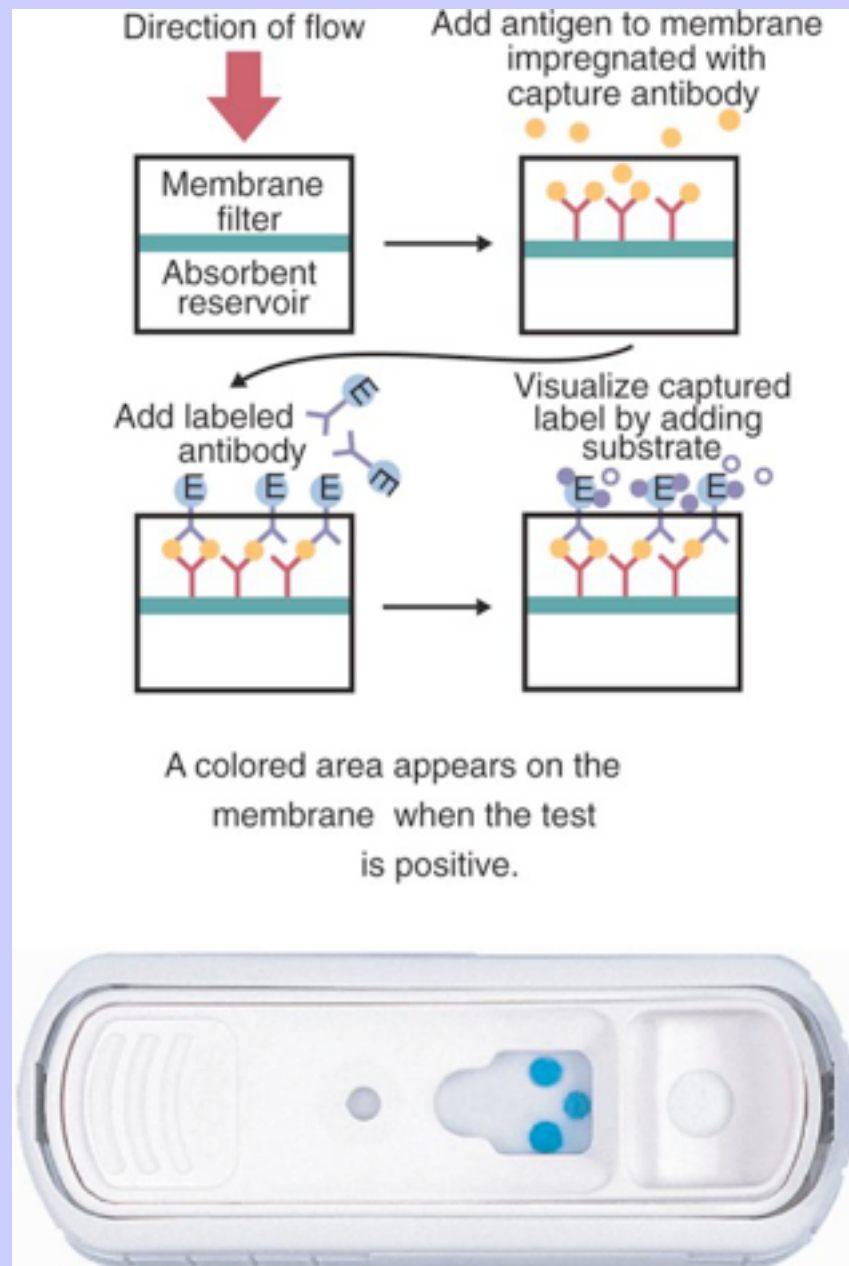
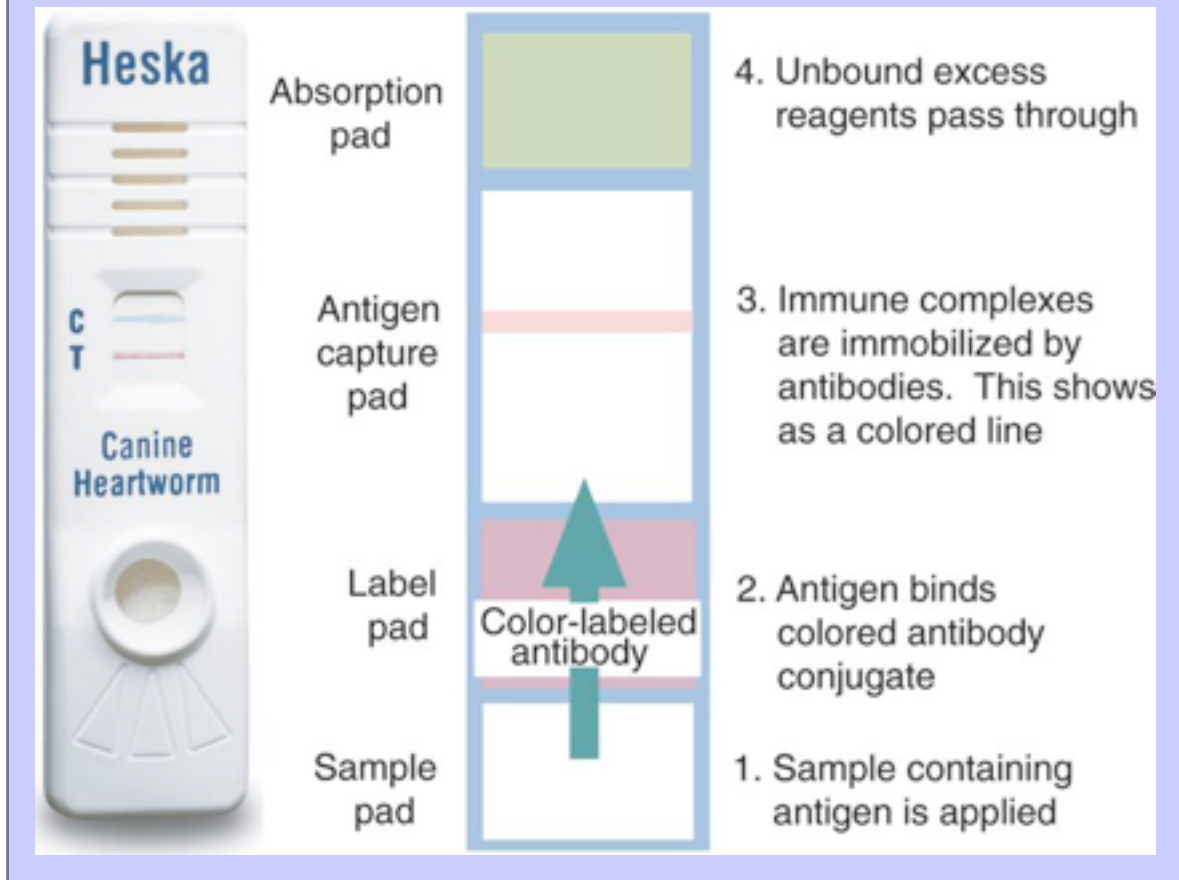


FIGURE 38-14 Immunochromatography. A sample containing antigen flows through a porous strip, and positive reactions are shown by the appearance of a colored band. (Courtesy Heska, Inc.)



38.7.2 Immunochromatography

To make assays even faster and easier to read, immunochromatography assays are being increasingly employed. In their simplest form, these involve allowing an antigen solution (such as infected blood) to flow through a porous strip. As the solution passes through the strip, it first flows through a zone where it meets and solubilizes dried labeled antibody and forms immune complexes. The antibody may be labeled with either colloidal gold (pink color) or colloidal selenium (blue color). The fluid then flows through a detection zone containing immobilized antibody against the antigen. This captures any immune complexes. In a positive test, a pink or blue line develops in the detection zone ([Figure 38-14](#)). This simple procedure permits multiple samples to be analyzed in a simple one-step procedure. A positive control band can be developed as well and the use of an effective prefilter can permit the use of whole blood. This assay is used for the detection of heartworm or feline leukemia antigens.

Immunochromatography systems are made in several different formats. For example, the sample containing the antigen of interest can be applied to a porous membrane at one end of the strip. Then capillary action can draw

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the solution through a conjugate pad, a solid-phase detection zone, and into an absorption pad. Buffer may be added to speed the flow of antigen solution. In another form of this assay, the antigen solution is dropped onto a pad containing antibody. This is followed by wash buffer that drives the immune complexes through the pad to an area containing labeled antiglobulin. The immune complexes are captured at this point. Then buffer can be applied at the other end of the pad and used to flush labeled complexes back to the detection zone, where they form a colored band.

38.8 ANTIBODY LABELS

Although radioisotopes and enzymes are commonly used as labels for primary binding tests, both have disadvantages. For example, radioactive isotopes may have a short half-life, are potentially hazardous, and may require expensive detection devices. Enzymes, though stable and relatively cheap, are large molecules that may inhibit antibody activity or lose enzymatic activity in the process of being bound to antiglobulin. One alternative is to use the small molecule biotin and its specific binding protein, avidin. Biotin can bind to proteins without affecting their biological activity. Avidin binds very strongly and specifically to biotin and may be conjugated with enzymes.

The most popular enzymes used in ELISAs include alkaline phosphatase, horseradish peroxidase, and β -galactosidase. Enzyme assays involving the production of luminescent products, such as luciferase, may be many times more sensitive than conventional enzyme assays but require sophisticated instruments to measure the luminescence produced. Colored dyes linked to antibodies have been used in dipstick assays. Reagents linked to ferritin or colloidal gold may be used to identify the location of antigens in cells examined by electron microscopy because such labels are electron dense. As described above, colloidal gold and colloidal selenium are colored and so can be used as labels in simple immunochromatography tests.

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38.9 THE FLOW CYTOMETER

Because of the importance of identifying cell immunophenotypes, considerable effort has gone into developing rapid identification methods for cell surface antigens. Immunophenotypes can now be automatically analyzed in great detail and with high efficiency using a flow cytometer ([Figure 38-15](#)). In this instrument a suspension of cells is pumped through a very narrow tube so that the cells pass through in single file. A laser beam is directed through the cell stream, and the effects of each cell on the light beam are observed. Thus the scatter of the light beam in a forward direction can be used to give a measure of a cell's size. The light scattered to the side by a cell gives a measure of a cell's surface roughness and internal complexity. A combination of these two parameters can be used to identify all the leukocytes in a blood sample.

The flow cytometer can, however, be used to measure much more than this. Thus if a cell suspension is mixed with a monoclonal antibody bound to a fluorescent dye, the labeled antibody will bind only to cells carrying the appropriate antigen on its surface. This subpopulation can be characterized and counted ([Figures 38-16](#) and [38-17](#)). By using antibodies labeled with different colored fluorescent dyes, the expression of multiple cell surface antigens can be analyzed simultaneously. It is possible to use the flow cytometer to follow sequential changes in the immunophenotype of mixed-cell populations ([Figure 38-18](#)).

38.10 SECONDARY BINDING TESTS

The reactions between antigens and antibodies are commonly followed by a secondary reaction. Thus if antibodies combine with soluble antigens in solution, the resulting complexes may precipitate. Antibodies binding to

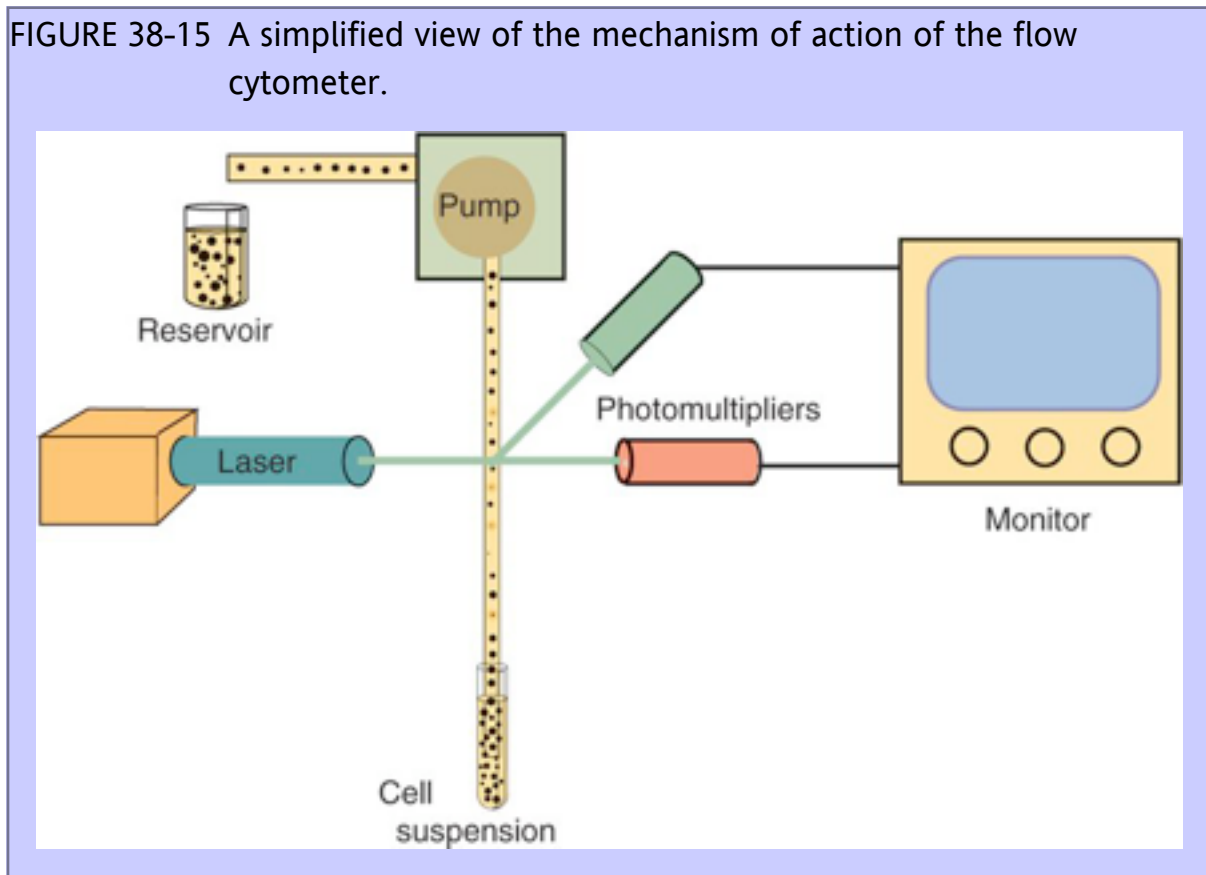
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particulate antigens (e.g., bacteria or red blood cells), may make them clump or agglutinate. If an antibody can activate the classical complement pathway and the antigen is on a cell surface, then cell lysis may result. These reactions can be employed in many different serological assays.

38.1 PRECIPITATION TESTS

If a solution of soluble antigen is mixed with a strong antiserum, the mixture becomes cloudy within a few minutes and then flocculent. Within an hour a precipitate consisting of antigen-antibody complexes settles to the bottom of the tube. Increasing amounts of soluble antigen can be mixed with a constant amount of antibody, and the amount of precipitate that develops will indicate the relative proportions of the reactants. No obvious precipitate is formed at low antigen concentrations. As the amount of antigen increases, larger quantities of precipitate form until the amount is maximal. However, with the addition of yet more antigen, the amount of precipitate gradually diminishes,

FIGURE 38-15 A simplified view of the mechanism of action of the flow cytometer.



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FIGURE 38-16 How an immunoglobulin bound to a fluorescent dye can be used to identify cell surface cluster of differentiation (CD) molecules in a flow cytometer.

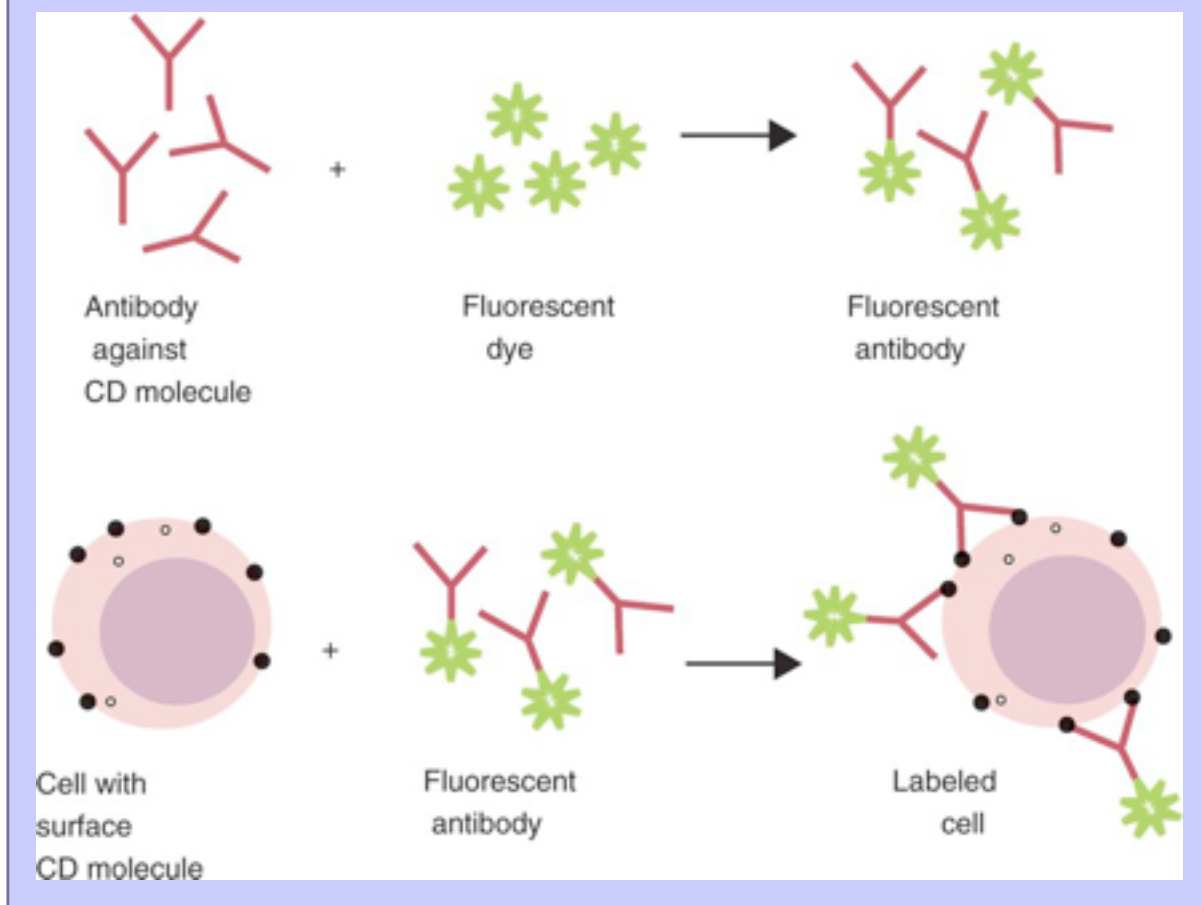


FIGURE 38-17 A typical flow cytometer readout from labeling a cell population with antiequine CD4. The intensity of fluorescent labeling increases from left to right. Thus unlabeled control cells form the unshaded left peak. When a mixture of CD4⁺ and CD4⁻ cells is examined, it forms two distinct peaks (*shaded area*). The left peak consists of unlabeled (CD4⁻) cells. The right peak consists of labeled (CD4⁺) cells. The area under each peak is a measure of the size of each cell subpopulation. (Courtesy Dr. R.R. Smith III.)

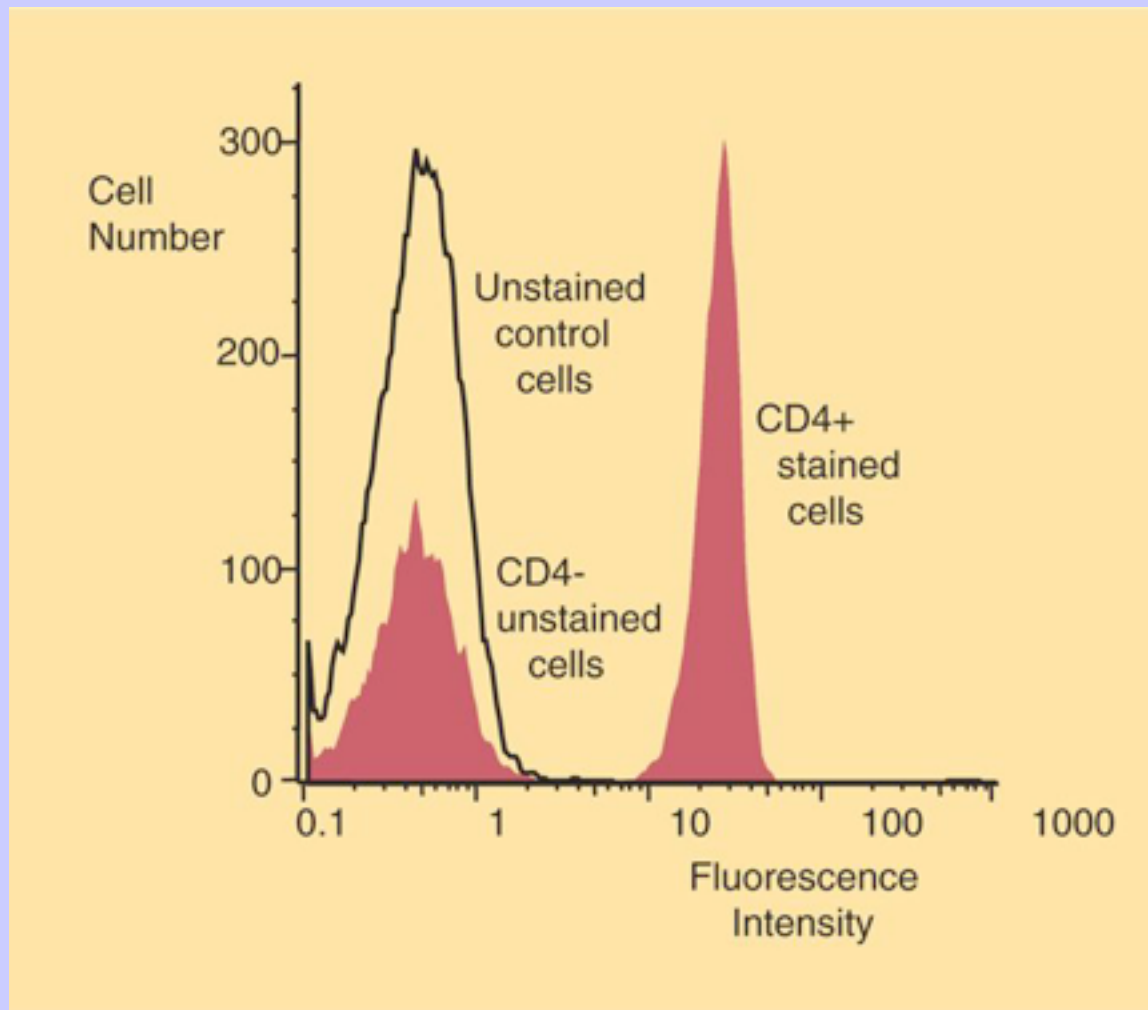
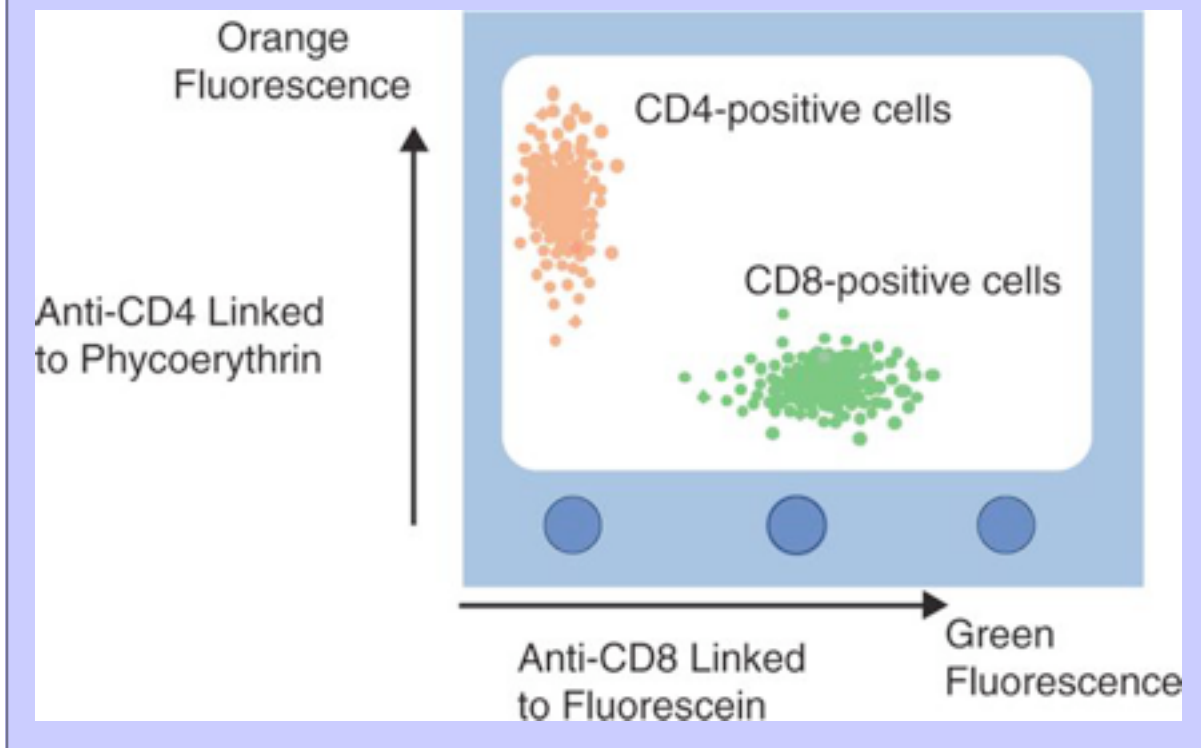


FIGURE 38-18 The pattern seen on a flow cytometer screen when analyzing lymphocyte populations stained with two different fluorescence-conjugated antibodies. It is usual to label one population with a green dye and the second population with a red or orange dye.



until none is observed in tubes containing a large excess of antigen ([Figure 38-19](#)). Equine IgG3 antibodies behave in a somewhat different fashion, producing a distinct flocculation over a very narrow range of antigen concentrations ([Figure 38-20](#)).

In the first stage of these reactions, only a little antigen is complexed to antibody and little precipitate is deposited. In the tubes where most precipitation occurs, both antigen and antibody are completely complexed and neither can be detected in the supernatant fluid. This is called the equivalence zone, and the ratio of antibody to antigen is optimal. When antigen is added to excess, little precipitate is formed, although soluble immune complexes are present and free antigen may be detected in the supernatant fluid.

This result is due to the fact that antibodies are usually bivalent and therefore can cross-link only two epitopes at a time, but complex antigens are generally

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FIGURE 38-19 The effect of mixing increasing amounts of antigen (bovine serum) with a constant amount of antibody (rabbit antiserum). The tube with the greatest amount of precipitate is the one in which the ratio of antigen to antibody is optimal. A quantitative precipitation curve of this test shows this effect graphically.

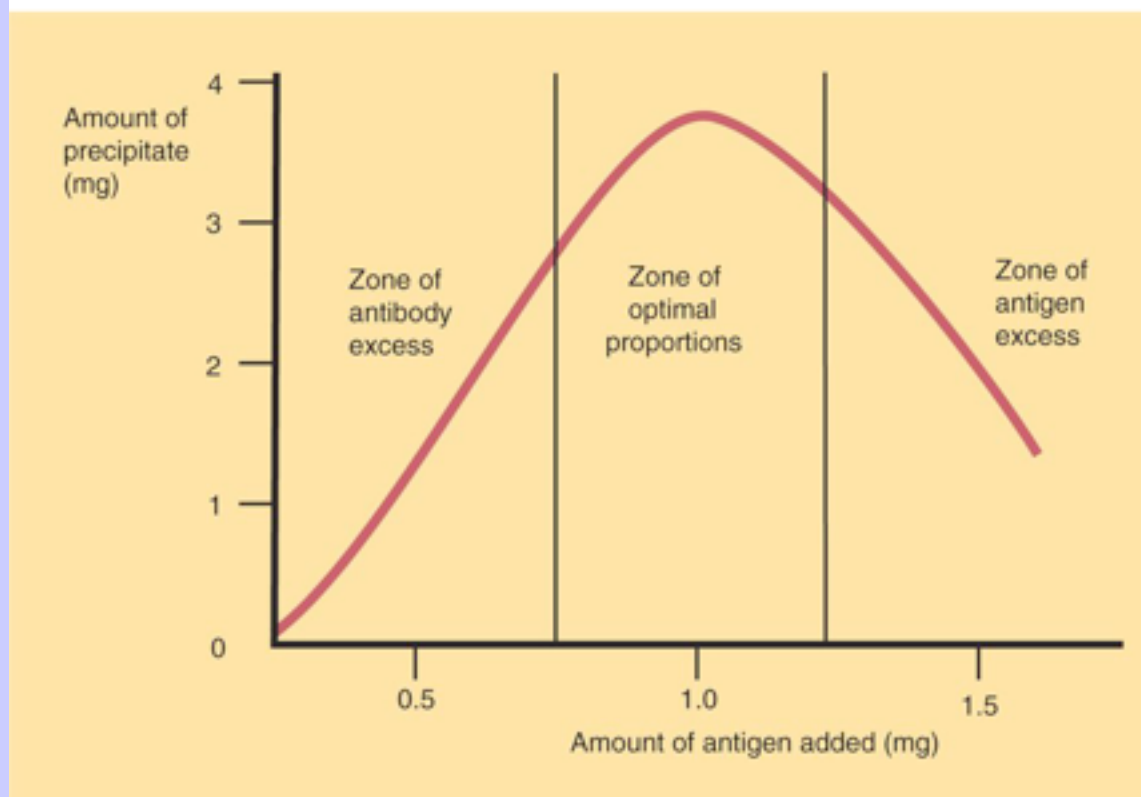
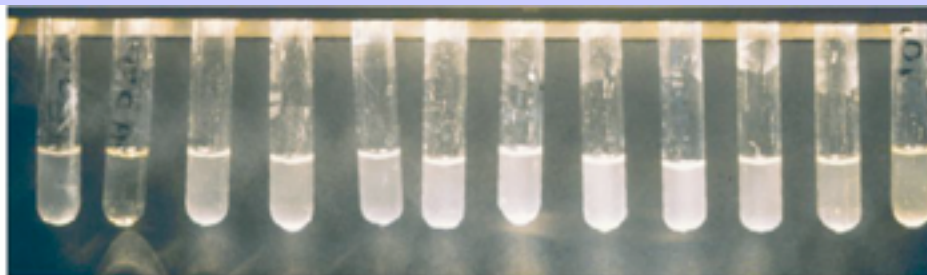
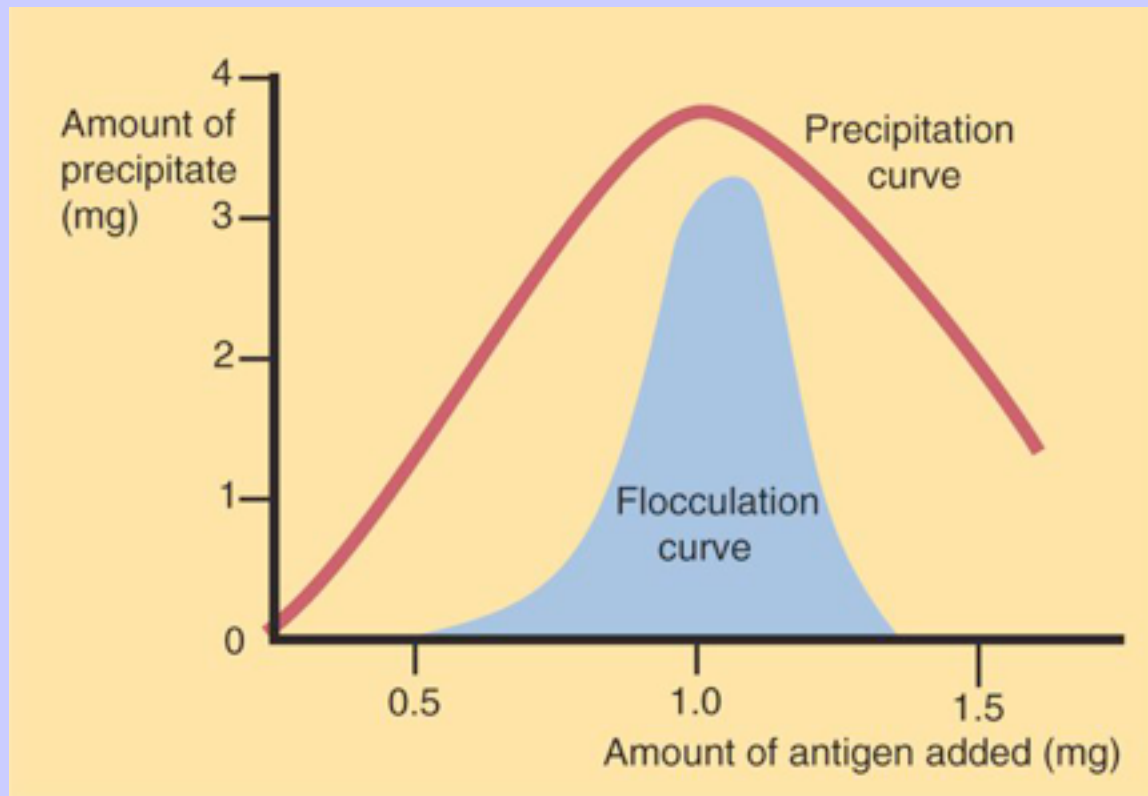


FIGURE 38-20 A quantitative precipitation curve of the type obtained when horse serum is used as a source of antibody. Flocculation occurs only over a narrow range of antigen-antibody mixtures.



multivalent, possessing many epitopes ([Figure 38-21](#)). Where there is excess antibody, each antigen molecule is covered with antibody molecules, preventing cross-linkage and thus precipitation. When the reactants are in optimal proportions, the ratio of antigen to antibody is such that cross-linking and lattice formation are extensive. As this lattice grows it becomes insoluble and eventually precipitates. In mixtures where antigen is in excess, each antibody molecule binds two antigen molecules. Further cross-linkage is impossible, and since these complexes are small and soluble, no precipitation occurs. Mononuclear phagocytes are most efficient at binding and removing complexes formed at optimal proportions and in antibody excess. Small immune complexes formed in antigen excess are poorly removed by phagocytic cells but are deposited in vessel walls and in glomeruli, where they cause damage classified as type III hypersensitivity (see [Chapter 27](#)).

38.11.1 Immunodiffusion

One simple method of demonstrating precipitation of antigen by antibody is immunodiffusion or gel diffusion. Round wells, about 5 µm in diameter and about 1 cm apart, are cut in a layer of clear agar. One well is then filled with soluble antigen and the other with antiserum; the reactants diffuse out radially. Where

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FIGURE 38-21 The mechanism of immunoprecipitation. In both antigen and antibody excess, small, soluble, immune complexes are produced. However, at optimal proportions, large insoluble complexes are generated.

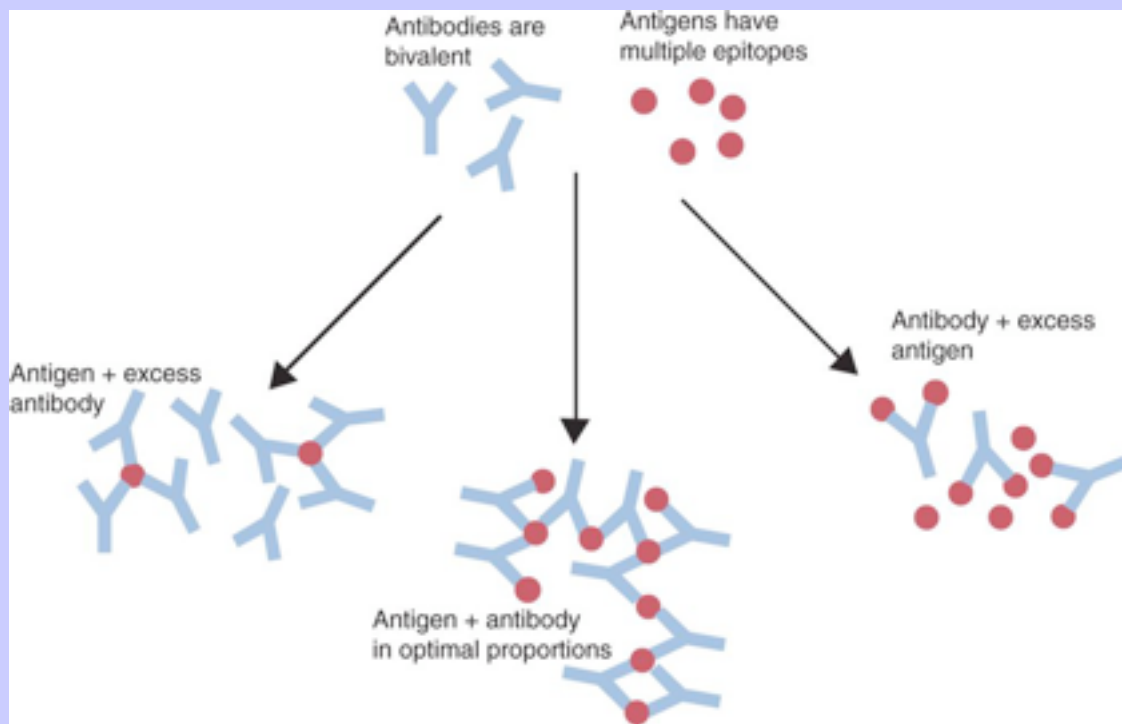
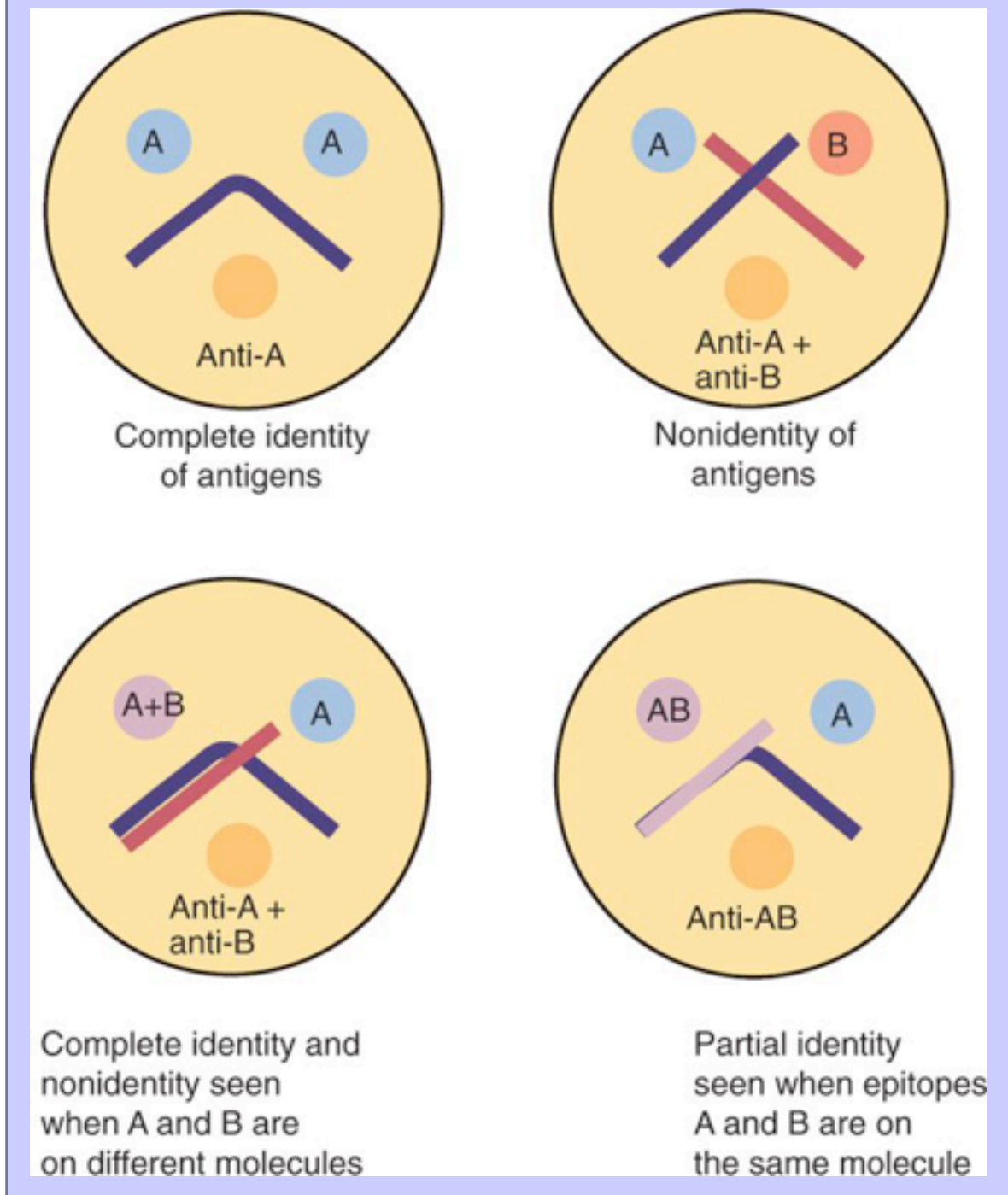


FIGURE 38-22 Precipitation in agar gel. Antigen and antibody diffusing from their respective wells precipitate in a region where optimal proportions are achieved. In this example, the antigen is identical in both top wells. As a result, the precipitation lines fuse to show complete identity.



FIGURE 38-23 The gel diffusion technique to determine the relationship of two antigens.



the reactants meet in optimal proportions an opaque white line of precipitate appears ([Figure 38-22](#)).

If the solutions used contain several different antigens and antibodies, the components are unlikely to reach optimal proportions in exactly the same position. Consequently, a separate line of precipitate is produced for each interacting set of antigens and antibodies. This test can be used to determine the relationship between antigens. If two antigen wells and one antibody well are set up as in [Figures 38-22](#) and [38-23](#), then lines will form between each antigen well and the antibody well ([Figure 38-23](#)). If these two lines join, the two antigens are probably identical. If the lines cross, then the two antigens are completely different. If the lines merge with spur formation, a partial identity exists, indicating that one antigen possesses epitopes not present in the other.

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The Coggins test is a gel diffusion method used to detect antibodies against equine infectious anemia virus in horse serum. In this test an extract of infected horse spleen or a cell culture antigen reacts with the serum of the horse under test in agar gel, and the development of a line of precipitate constitutes a positive reaction. A similar test is used to identify cattle infected with bovine leukemia virus.

38.11.1.1 Radial Immunodiffusion

If an antigen solution diffuses into agar in which specific antiserum has been incorporated, a ring of precipitate will form around the antigen well. The area of this ring is proportional to the amount of antigen in the well. A standard curve may therefore be constructed using known amounts of antigen ([Figure 38-24](#)). Unknown solutions of antigen can then be accurately assayed by comparing the ring diameters they give rise to with the standard curve.

38.11.2 Immunelectrophoresis and Related Techniques

Although conventional gel-diffusion techniques give a separate precipitation line for each antigen-antibody system in a mixture, it is often difficult to resolve all the components in a complex mixture. One way to improve the resolution of the system is to first separate the antigen mixture by electrophoresis before undertaking immunodiffusion. This technique is called immunelectrophoresis, and it is used to identify proteins in body fluids ([Figure 38-25](#)).

Immunelectrophoresis involves the electrophoresis of the antigen mixture in agar gel in one direction. A trough is then cut in the agar parallel to this line of separated proteins. Antiserum against the whole serum is placed in this trough and allowed to diffuse laterally. When the diffusing antibodies encounter antigen, curved lines of precipitate are formed. One arc of precipitation forms for each of the constituents in the antigen mixture. This technique, which can resolve the proteins of normal serum into 25 to 40 distinct precipitation lines ([Figure 38-26](#)), is used to identify the absence of a normal serum protein, such as occurs in animals with a congenital deficiency of some complement components. It is also used to detect the presence of excessive amounts of an individual component, as in animals with myeloma (see [Chapter 13](#), [Figure 13-23](#)).

If, instead of being permitted to passively diffuse into agar-containing antiserum as in the radial immunodiffusion technique, the antigen is driven into the antiserum agar by electrophoresis, then the ring of precipitation around each well becomes deformed into a rocket shape. The length of the rocket is proportional to the amount of antigen placed in each well. This technique is called rocket electrophoresis.

FIGURE 38-24 A radial immunodiffusion assay. The area of precipitation is proportional to the concentration of antigen. In this case, antiserum to bovine immunoglobulin A (IgA) is incorporated in the agar and is used to measure bovine serum IgA levels.

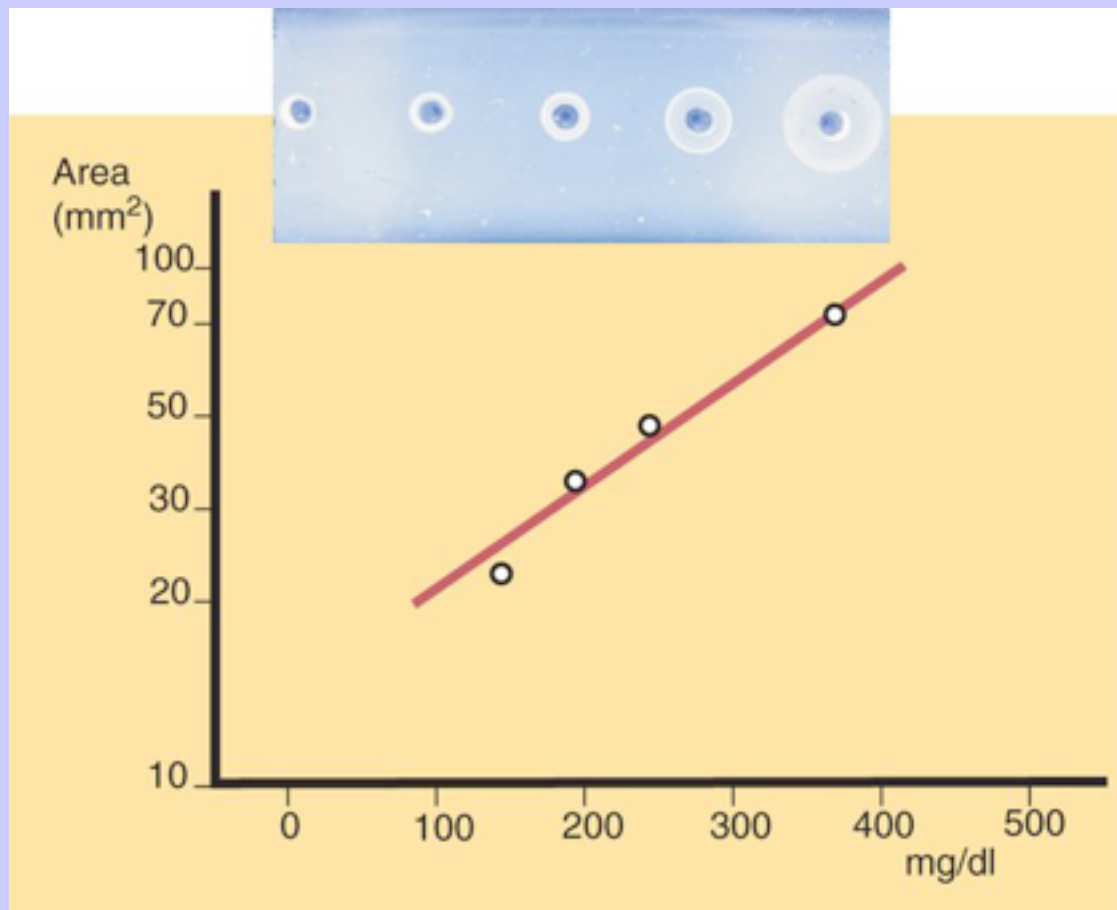


FIGURE 38-25 The technique of immunoelectrophoresis (see text for details).

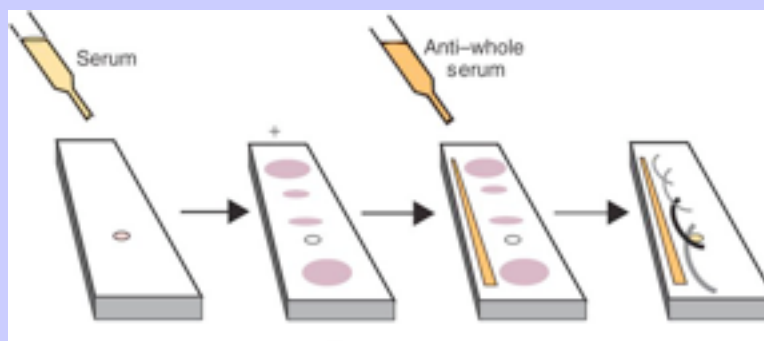


FIGURE 38-26 Immunelectrophoresis of pig serum showing the lines of precipitation produced by some of the major serum proteins (see also [Chapter 13, Figure 13-23](#)).

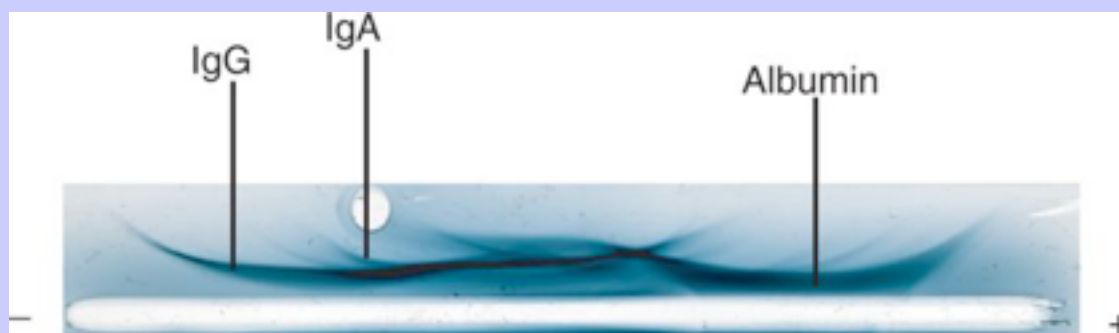


FIGURE 38-27 The principle of antibody titration. Serum is first diluted in a series of tubes. A constant amount of antigen is then added to each tube and the tubes are incubated. At the end of the incubation period, the last tube in which a reaction has occurred is identified. In this example, agglutination has occurred in all tubes up to a serum dilution of 1 : 8. The agglutination titer of the serum is said to be 8.

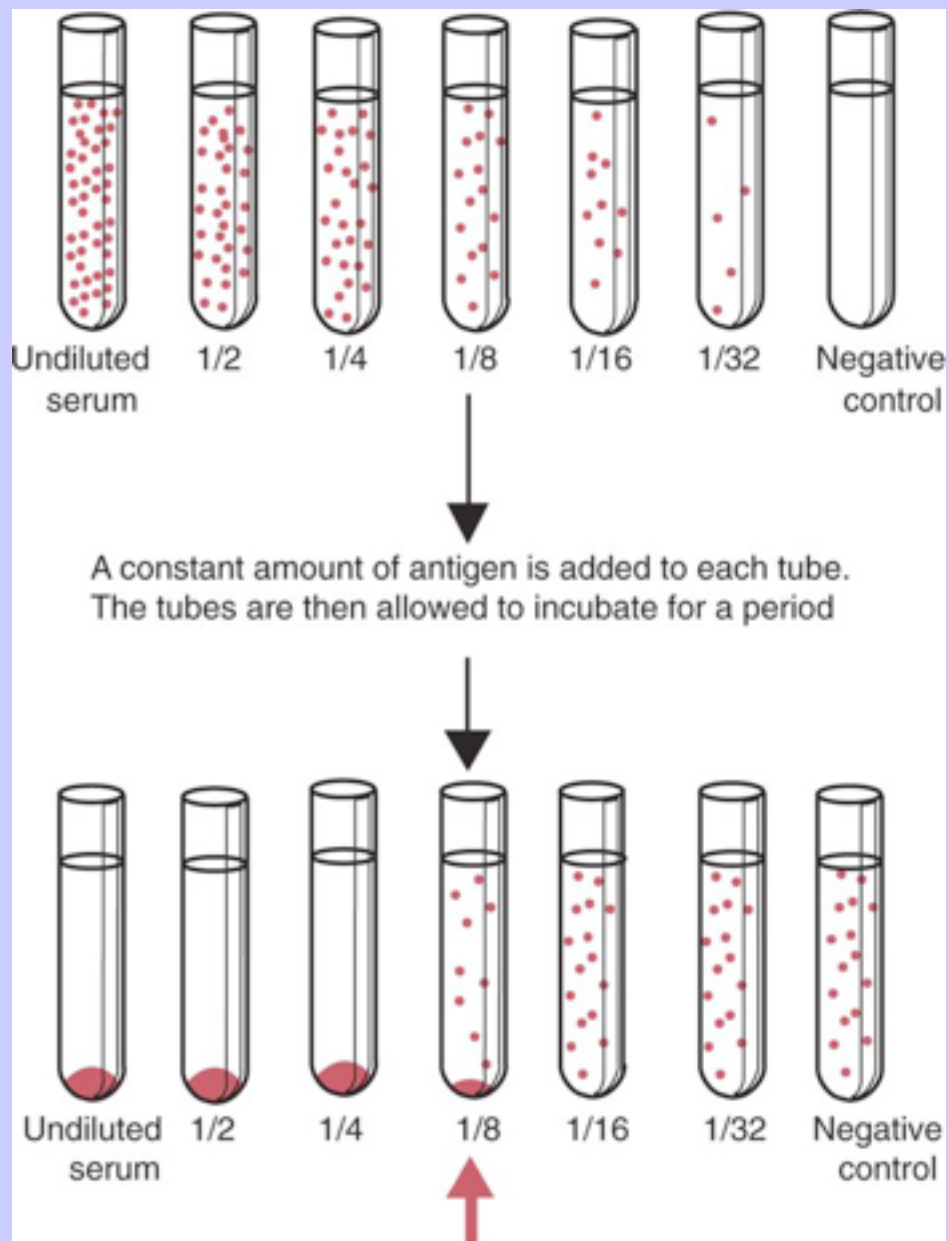


Table 38-2 Role of Specific Immunoglobulin Classes in Serological Assays

Property	IgG	IgM	IgA	Eq IgG3
Agglutination	+	+++	+	–
Complement activation	+	+++	–	–
Precipitation	+++	+	±	±
Time of appearance (days)	3–7	2–5	3–7	3–7
Time to peak titer (days)	7–21	5–14	7–21	7–21

38.12 TITRATION OF ANTIBODIES

Although the simple detection of antibodies or antigen is sufficient for many purposes, it is usually desirable to quantitate the reaction. One way of measuring specific antibody levels is by titration. The serum under test is diluted in a series of decreasing concentrations ([Figure 38-27](#)). Each dilution is then tested for activity. The reciprocal of the highest dilution giving a positive reaction, called the titer, provides an estimate of the amount of antibody in that serum.

38.13 AGGLUTINATION

Because antibodies are bivalent they can cross-link particulate antigens such as bacteria or foreign red cells, resulting in their clumping or agglutination. Antibodies differ in their ability to cause agglutination; for example, IgM antibodies are more efficient than IgG antibodies ([Table 38-2](#)). If excess antibody is added to a suspension of antigenic particles, then, just as in the precipitation reaction, each particle may be so coated by antibody that agglutination is inhibited. This lack of reactivity at high concentrations of antibody is termed a prozone. Another cause of prozone formation is the presence of antibodies that cannot cause agglutination. These nonagglutinating antibodies are also called incomplete antibodies. The reason for their lack of agglutinating activity is not completely understood; one possibility is that the epitopes with which they react lie deep within the surface coat of the particle, so deep that cross-linking cannot occur. An alternative suggestion is that they are capable of only restricted movement in their hinge region, causing them to be functionally monovalent.

38.13.1 Antiglobulin Tests

If it is necessary to test for the presence of nonagglutinating antibodies on the surface of particles such as bacteria or erythrocytes, then a direct antiglobulin test may be used. The washed particles are mixed with an antiglobulin; if antibodies are present on their surface, agglutination will occur ([Figure 38-28](#)).

38.13.2 Passive Agglutination

Since agglutination is a much more sensitive technique than precipitation, it is sometimes useful to convert a precipitating system to an agglutinating one ([Figure 38-29](#)). This may be done by chemically linking soluble antigen to inert particles such as erythrocytes, bacteria, or latex. Erythrocytes are among the best particles

FIGURE 38-28 The direct antiglobulin test. The presence of the antiglobulin is required to agglutinate particles coated with nonagglutinating antibody.

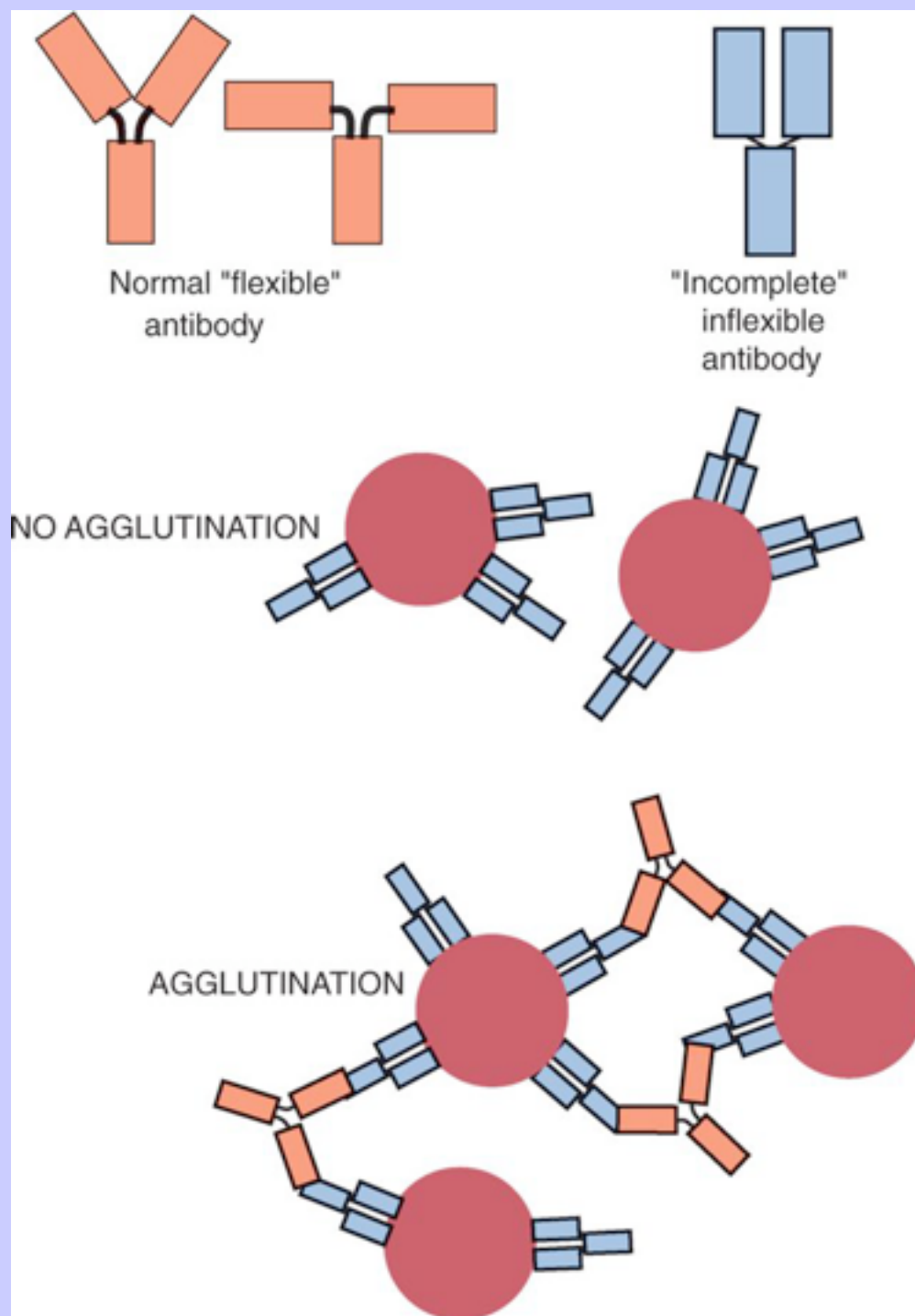


FIGURE 38-29 The relationship between precipitation and agglutination. This is essentially a consequence of the size of the antigenic particle. Large particles agglutinate. Small particles and soluble molecules precipitate.

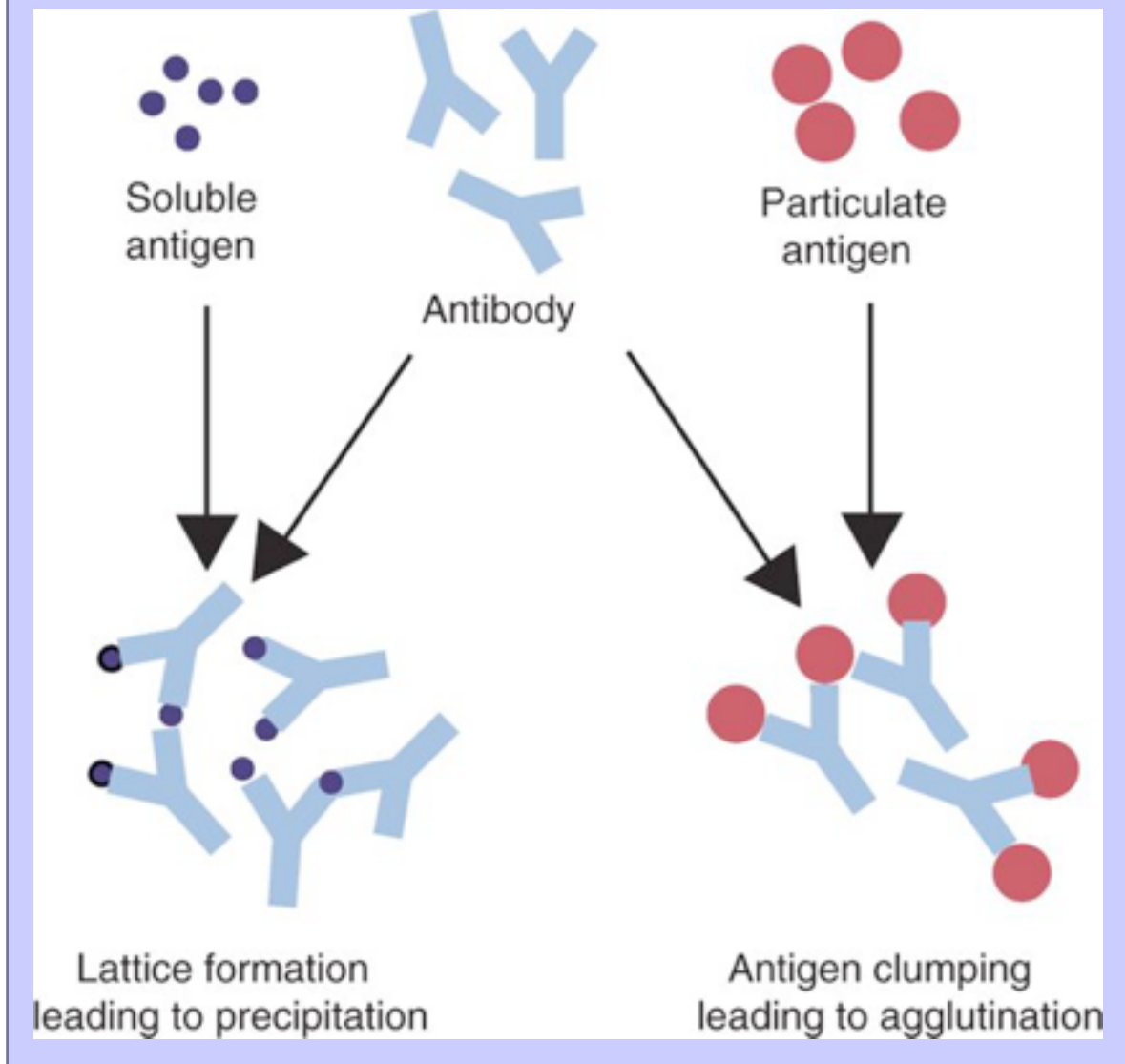
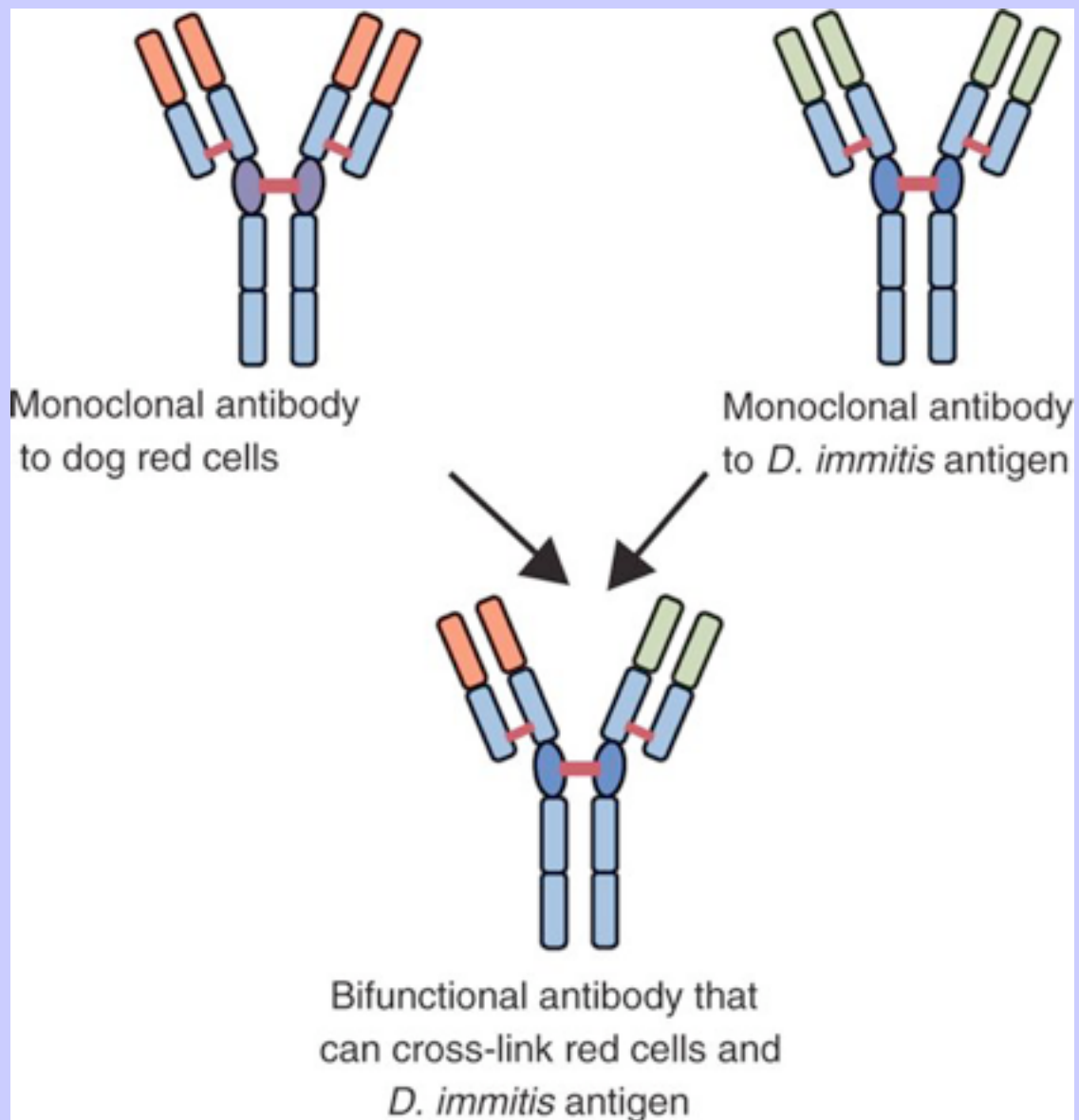


FIGURE 38-30 The use of bifunctional antibodies to cross-link two different epitopes. In this example the antibody cross-links canine red cells with heartworm antigens. If the antibody is mixed with infected dog blood, it will cause visible hemagglutination.



for this purpose, and tests that employ coated erythrocytes are called passive hemagglutination tests.

An interesting variant of hemagglutination tests is the use of bifunctional monoclonal antibodies. A bifunctional monoclonal antibody can be made by breaking the bonds between the two heavy chains so that two identical halves are formed. Then two halves from different immunoglobulins are then joined to produce a molecule that can cross-link two different epitopes. For example, a bifunctional antibody can be made where one antigen-

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binding site is directed against canine red blood cells and the other against adult heartworm (*Dirofilaria immitis*) antigen (Figure 38-30). When this reagent is mixed with whole blood from a heartworm-infected dog, it cross-links the heartworm antigen to the red cells, resulting in visible hemagglutination within a few minutes.

38.14 VIRAL HEMAGGLUTINATION AND ITS INHIBITION

Some viruses can bind and agglutinate mammalian and avian red cells. This virus-induced hemagglutination can help characterize an unknown virus. Inhibiting viral hemagglutination by antibody makes it possible to identify a specific virus or to measure antibody levels in serum. Hemagglutinating organisms include orthomyxoviruses and paramyxoviruses, alphaviruses, flaviviruses, and bunyaviruses as well as some adenoviruses, reoviruses, parvoviruses, and coronaviruses. They also include some mycoplasma such as *Mycoplasma gallisepticum*.

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38.15 COMPLEMENT FIXATION

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The activation of the classical complement pathway by antibody bound to antigen results in the generation of membrane attack complexes that can disrupt cell membranes. If the antibody is bound to red cells, these are ruptured and hemolysis occurs. In the complement fixation test this phenomenon is exploited to measure serum antibody levels.

Complement is a normal constituent of all fresh serum, but the complement in fresh, unheated guinea pig serum is the most efficient in hemolytic tests. Serum used as a source of complement for serological applications should be stored frozen in small volumes. Once thawed, it should be used promptly. It should not be repeatedly frozen and thawed.

The complement fixation test is performed in two parts. First, antigen and antibodies (the serum under test deprived of its complement by heating at 56° C) are mixed and incubated in the presence of normal guinea pig serum as a source of complement. After the antigen-antibody-complement mixture reacts, the amount of free complement remaining in the mixture is measured by adding an indicator system consisting of antibody-coated sheep red cells. Lysis of these cells (seen as the development of a transparent red solution) is a negative result because it indicates that complement was not activated and that antibody was absent from the serum under test (Figure 38-31). Absence of lysis (seen as a cloudy red cell suspension), which indicates that complement was consumed (or fixed), is a positive result. It is usual to titrate the serum being tested so that, if antibodies are present in that serum, as it is diluted the reaction in each tube will change from no lysis (positive) to lysis (negative). The titer is the highest dilution of serum in which no more than 50% of the red cells are lysed.

38.15.1 Cytotoxicity Tests

Complement may cause membrane damage, not only to erythrocytes but also to nucleated cells and to protozoa. Antibodies against cell surface antigens thus may be measured by reacting target cells with antibody and complement and estimating the resulting cell death. This form of assay has been employed to identify major histocompatibility complex class I molecules.

38.16 ASSAYS IN LIVING SYSTEMS

If an organism or antigen possesses biological activity, antibodies can be measured by their ability to neutralize this activity. The activities that may be neutralized include hemolysis of erythrocytes, lysis of nucleated cells, and disease or death in animals. Reactions such as these are highly variable because they tend to change gradually

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over a wide range of doses of organism or antigen. For this reason, results obtained from a single positive or negative neutralization test are usually meaningless. For example, 0.003 mg of tetanus toxin may kill some mice in a test group, but about five times that dose is required to kill all mice in the same group. In addition, the lowest dose of tetanus toxin that will kill all the animals in a group (the minimal lethal dose [LD]) will also be highly variable. It is equally difficult to estimate with precision the highest dose of toxin that will just fail to kill all test animals. The most dependable method of measuring the lethal effects of a toxin has been to estimate the dose that will just kill 50% of a group of test animals ([Figure 38-32](#)). In practice, it is usually not possible to arrive at this 50% end point by direct experimentation; it is usually necessary to calculate it by plotting the results against the dose of toxin given and arriving at the 50% end point by calculation.

The dose required to kill 50% of a group of experimental animals is called the LD₅₀. Similarly, the dose of complement that lyses just 50% of a red cell suspension is called the CH₅₀; the dose of organisms that infects 50% of animals is the ID₅₀; the dose that infects just 50% of tissue cultures is the TCID₅₀; and the dose of antiserum or vaccine that protects 50% of challenged animals is the PD₅₀.

FIGURE 38-31 The principle of the complement fixation test. Complement, if fixed by antigen and antibody, is unavailable to lyse the target cells in the indicator system. In the absence of antibody the complement remains unfixed and is available to lyse the indicator system.

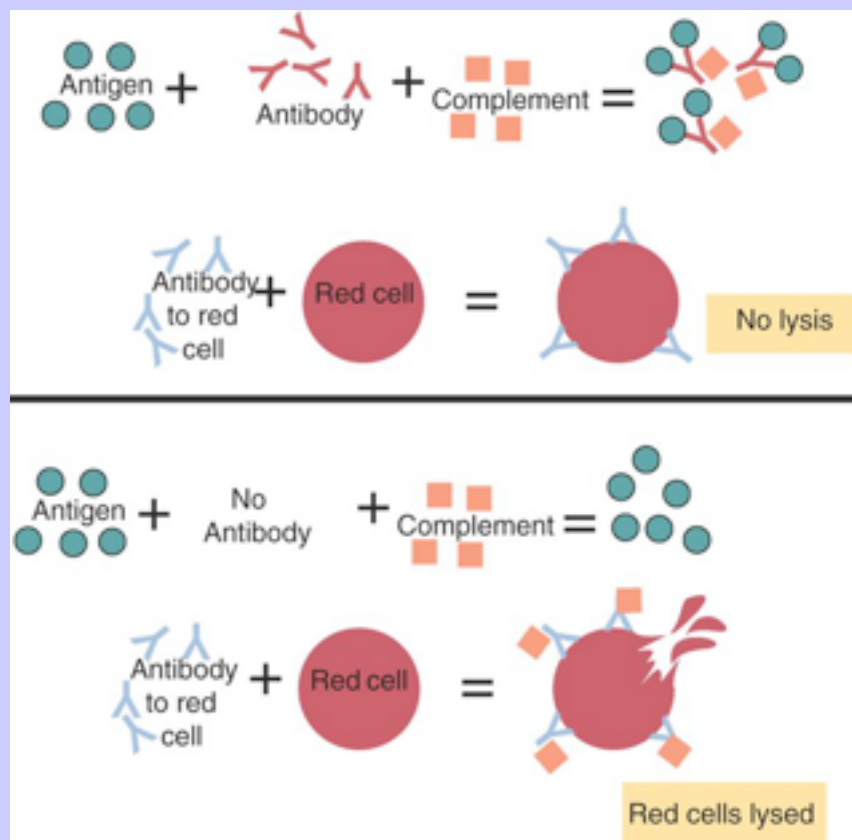
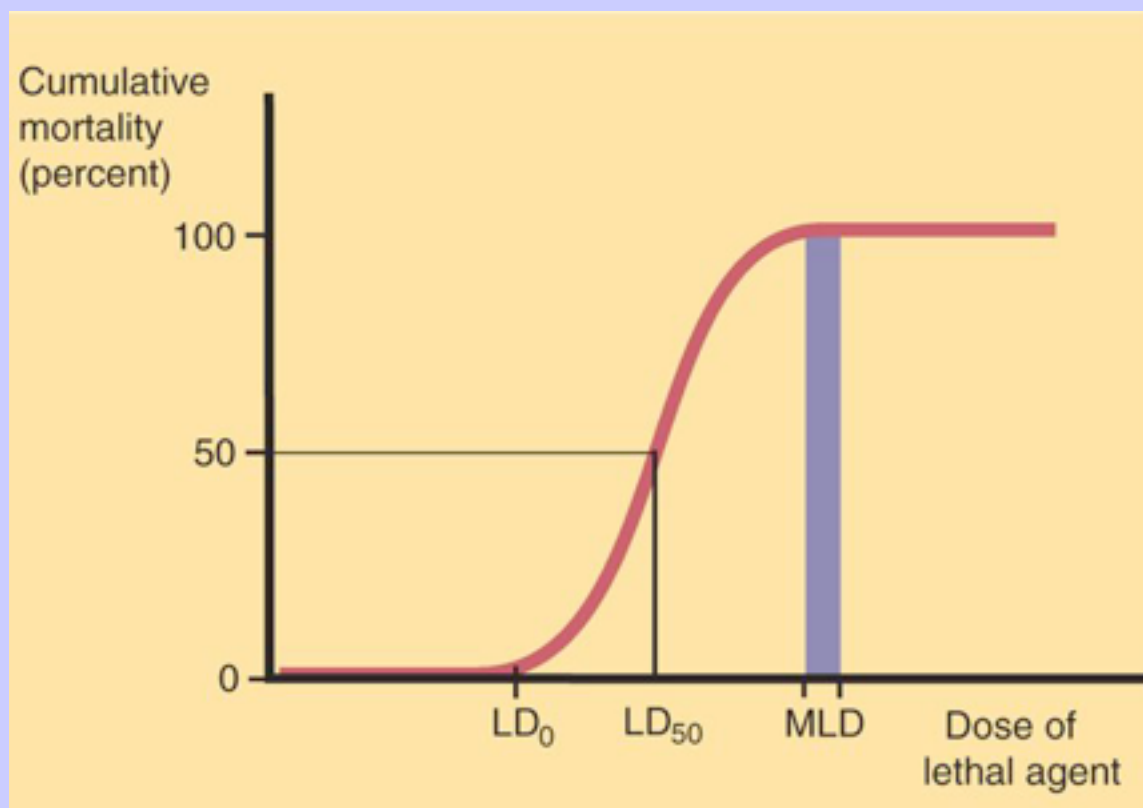


FIGURE 38-32 A cumulative mortality curve showing how the lethal dose 50 (LD_{50}) provides a more accurate estimate of the lethal effects of a toxin than either the LD_0 or the minimal lethal dose (MLD).



38.16.1 Neutralization Tests

Neutralization tests estimate the ability of antibody to neutralize the biological activity of antigen when mixed with it in vitro. These tests may be used to identify bacterial toxins such as *Clostridium perfringens* α -toxin or staphylococcal α -toxin.

Viruses may be prevented from infecting cells after specific antibody has combined with and blocked their critical attachment sites. This reaction is the basis of the neutralization tests that are employed either for the identification of unknown viruses or for the measurement of specific antiviral antibody. Neutralization tests are highly specific and extremely sensitive. Thus, antiserum to coliphage T4 will neutralize phage-induced lysis of *Escherichia coli* because antibodies can block the receptor on the phage tail, thus preventing its attachment to a bacterium. A single antibody molecule is sufficient to cause this blockage, and a phage neutralization test may therefore detect as little as 0.00005 mg of antibody.

38.16.2 Protection Tests

A protection test is a form of neutralization test carried out entirely in vivo. The protective properties of a specific antiserum are measured by administering it in increasing dilutions to a group of test animals, which may then be challenged with a standard dose of pathogenic organisms or toxin. Although protection tests provide a direct measure of the therapeutic efficacy of an antiserum, they are also subject to great experimental variation because of differences among animals. Thus animals differ in their susceptibility to infection and in a number of other factors, such as the rate of absorption of antiserum, the level of activity of the mononuclear phagocyte system, and the half-life of the passively administered immunoglobulin. As in neutralization tests, meaningful results can be obtained only if large numbers of animals are employed and if the challenge dose is carefully standardized. It is usual to use a dose of organisms or toxin containing a known number of LD₅₀ or ID₅₀.

Similarly, the protective effect of an antiserum may be expressed in PD₅₀, the dose required to protect 50% of a group of animals.

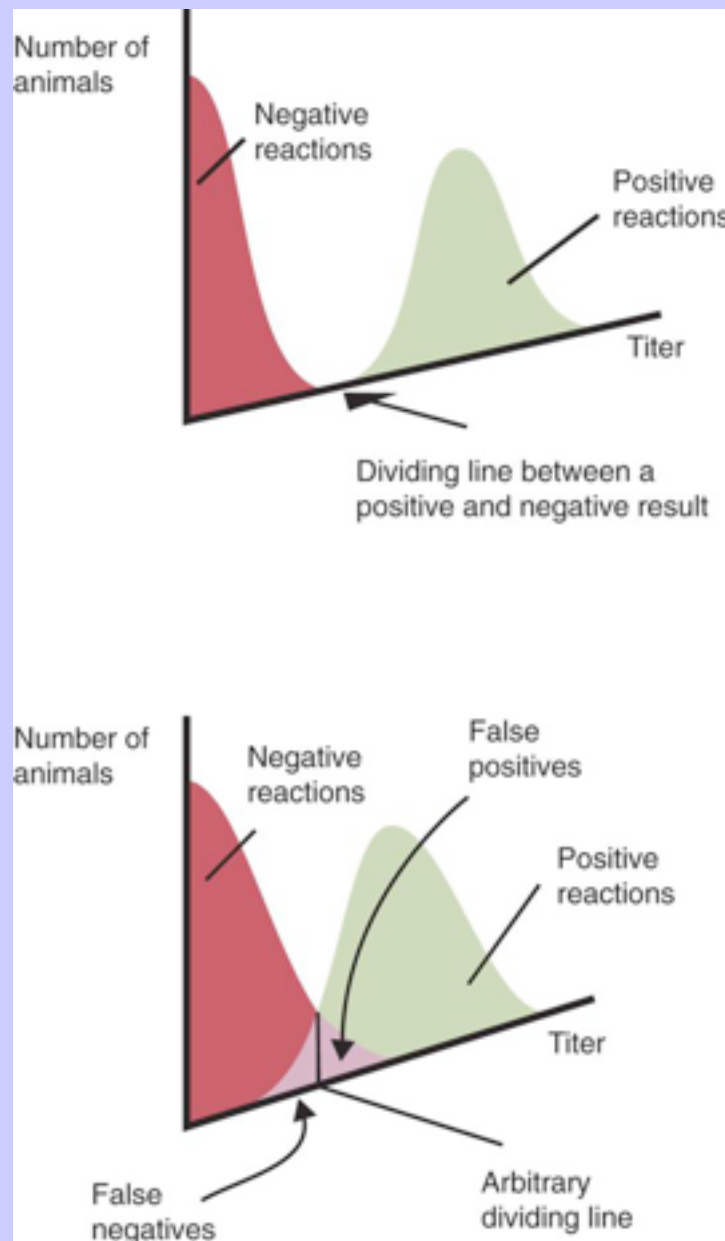
38.17 DIAGNOSTIC APPLICATIONS OF IMMUNOLOGICAL TESTS

Obviously, the presence of antibodies to a specific organism in an animal's serum indicates previous exposure to an epitope present on that organism. It does not, however, prove that infection exists or that any concurrent disease is actually caused by the organism in question. For example, the fact that the sera of most healthy horses contain antibodies to *Salmonella* serotype *typhimurium* does not prove that most horses are suffering from salmonellosis. Thus the presence of antibodies to an organism in a single serum sample is rarely of diagnostic significance. Only if at least two samples are taken 1 to 3 weeks apart and show at least a fourfold rise in titer can a diagnosis be made. This should be done only in conjunction with careful analysis of clinical factors.

A second feature that must be considered in the interpretation of serological tests is the possibility of errors. Technical errors are usually prevented by incorporation of appropriate controls into the test system. Other errors, however, are largely unavoidable. These may be of two types: false-positive results and false-negative results. A test in which a large proportion of the positive results is false is nonspecific, whereas one with a very high proportion of false-negative results is insensitive. In general, the level of such errors is set by the criteria used to differentiate positive from negative reactions ([Figure 38-33](#)). If these criteria are adjusted so that the number of false-positive results is reduced, there will usually be an increase in false-negative results and vice versa. Thus, highly sensitive tests tend to be relatively nonspecific and highly specific tests are generally insensitive. To find the right criteria for reading a test, which entails finding the optimal sensitivity and specificity, the requirements of the test procedure and the importance of false-positive and false-negative reactions must be taken into account. Ideally, the criteria used in interpreting the test results would be so obvious and absolute that each test would be absolutely sensitive and specific. Unfortunately, such ideal tests and conditions are uncommon.

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FIGURE 38-33 Schematic diagrams depicting the errors associated with immunological tests. The top diagram depicts an ideal test in which there is no ambiguity in interpreting test results. The bottom diagram depicts a more typical test in which an arbitrary line must be used to separate positive from negative results. Moving this dividing line changes the relative proportions of false-positive and false-negative results.



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As has been evident from the discussions earlier in this chapter, the advantages and disadvantages of each immunodiagnostic test vary according to the specific requirements of the investigator, the nature of the antigen employed, and the complexity, sensitivity, and specificity of each method. In general, the selection of a diagnostic test represents a compromise among its sensitivity, its specificity, and its complexity. The latter includes the number of steps, the time involved, the degree of technical expertise required, the cost, and the equipment needed to conduct the test. Although precise guidelines cannot be drawn, it is usually most appropriate to use the most sensitive and specific test that can be satisfactorily performed with the available technical assistance and equipment, at the lowest cost in the shortest possible time.

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Note: Of the 350 officially recognized CD molecules, many have no known function at this time while many others do not play a significant role in the immune system. This list summarizes the key features of only those CD molecules described in the text.

CD1

A family of class Id MHC-like molecules that are antigen-presenting molecules for lipids and glycolipids. Heterodimers of 49/12 kDa, they are found on thymocytes, macrophages, dendritic cells, and some B cells.

CD2

Also called LFA-2, this is a 67-kDa molecule found on T cells and some B cells. It is a cell adherence molecule whose ligands are CD58 (nonrodents) and CD48 (rodents only). CD2 was first recognized by its ability to bind sheep red blood cells to T cells to form characteristic rosettes.

CD3

The collective designation for the set of proteins that act as the signal transduction molecules of the TCR. They are 16- to 28-kDa glycoproteins found only on T cells.

CD4

A specific receptor for MHC class II molecules. It plays a key role in the recognition of processed antigen by helper T cells. It is a 59-kDa glycoprotein found on helper T cells, thymocytes, and monocytes. CD4 is also a receptor for HIV.

CD5

A 67-kDa receptor whose ligand is CD72. It is found on a subset of B cells and on all T cells. If blocked, T cells will no longer respond to antigen. Its expression on B cells varies among species. Thus it is found on all rabbit B cells and on a subpopulation of B cells (B-1a cells) in most species including mice and humans, but it is not found on B cells in rats or dogs.

CD8

This 32-kDa dimeric glycoprotein is a receptor for MHC class I molecules. It is expressed on cytotoxic T cells and plays a key role in the recognition of endogenous antigen by these cells.

CD9

A glycoprotein of 22 to 27 kDa expressed on platelets, immature B cells, eosinophils, basophils, and activated T cells.

CD10

An endopeptidase of 100 kDa expressed on T and B cell precursors.

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CD11

Also called LFA-1, this is a 180-kDa integrin α chain found on leukocytes. Three forms are known: 11a, 11b, and 11c. They play a key role in binding leukocytes to vascular endothelium.

CD14

This is a 55-kDa protein found on macrophages and granulocytes. It is the receptor for lipopolysaccharide-binding protein and therefore regulates the biological activities of this molecule.

CD15

This is a complex carbohydrate called Lewis-X. It is found on many cells, especially granulocytes. Its sialylated form, sialyl Lewis^x, is characteristic of NK cells. Its ligand is the selectin CD62.

CD16

Also called Fc γ RIII, this is a low-affinity receptor for IgG and for CD4. It is a 50- to 65-kDa dimer found on NK cells, granulocytes, and macrophages.

CD18

This is the integrin β 1 chain. A protein of 95 kDa, it is found on all leukocytes. It associates with the various forms of CD11. A mutation in the CD18 gene is responsible for leukocyte adherence deficiency in calves.

CD19

A glycoprotein of 95 kDa expressed on B cells and their precursors but not on plasma cells. It is also expressed on dendritic cells. It is associated with CD21. CD19 plays a key role in regulating the B cell response to antigen.

CD21

A complement receptor also called CR2. It is a glycoprotein of 145 kDa found on B cells, some T cells, and dendritic cells. Its several ligands include CD23 and C3d. CD21 regulates B cell responses in association with CD19.

CD22

Also called siglec-2, this is a B cell inhibitory receptor.

CD23

A receptor for IgE also called Fc ϵ R2, this is a glycoprotein of 45 kDa found mainly on mature B cells. In its soluble form it regulates the production of IgE. It can also regulate B cell responses by binding to CD21.

CD25

The α chain of the IL-2 receptor. A glycoprotein of 55 kDa, CD25 associates with the IL-2R β chain (CD122). It is expressed on activated T cells, B cells, and monocytes. CD25 expression is a feature of regulatory T cells.

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CD28

The ligand for both CD80 and CD86 expressed on activated B cells and other antigen-presenting cells. It is a homodimer of 45-kDa subunits found on most T cells. It plays a key role in T cell co-stimulation.

CD29

The β_1 integrin chain, a protein of 130 kDa expressed on leukocytes and platelets. In conjunction with its α chain (one of the forms of CD49), it binds these cells to extracellular matrix proteins.

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CD31

CD31 mediates adhesion between cells that express CD31 (e.g., leukocytes to endothelial cells) in a homophilic manner. (CD31 binds CD31 on the apposing cell.) It regulates the phagocytosis of dead and dying cells.

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CD32

A 40-kDa medium-affinity IgG receptor also called Fc γ R2, different forms of which are expressed on macrophages, granulocytes, and B cells.

CD33

Siglec-3, a molecule expressed on immature hematopoietic stem cells. It has carbohydrate-binding properties.

CD34

Also called sialomucin, CD34 is a 105- to 120-kDa glycoprotein expressed on endothelial cells, where it is a ligand for certain integrins.

CD35

A 160- to 250-kDa glycoprotein expressed on granulocytes, monocytes, B cells, NK cells, and primate erythrocytes. It is the receptor for the complement components C3b and C4b, so it is also called CR1. This molecule is important in opsonization and the removal of immune complexes from the body.

CD36

An 88-kDa receptor expressed on many different cell types. It serves as a pattern-recognition receptor and binds many different ligands, especially lipids. CD36 on intestinal γ/δ T cells binds bacterial lipoteichoic acids and helps trigger innate responses.

CD40

A 50-kDa member of the tumor necrosis factor receptor superfamily expressed on all antigen-presenting cells. Binding to its ligand CD40L (CD154) on activated helper T cells is essential for a successful antibody response and class switching.

CD41

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An integrin α chain of 120 kDa expressed on platelets and macrophages. It associates with its β chain (CD61) and binds to fibrinogen.

CD43

Also called sialophorin or leukosialin, this 115-kDa glycoprotein is expressed on all T cells as well as granulocytes, macrophages, NK cells, platelets, and activated B cells. It serves as an antiadhesive molecule on leukocytes.

CD44

A 90-kDa glycoprotein expressed in large amounts on T and B cells, monocytes, and granulocytes, as well as a wide variety of other cells. It is the principal receptor for hyaluronic acid. As a result it mediates binding of these cells to high endothelial venules.

CD45

A family of 190- to 220-kDa glycoproteins found on all cells of hematopoietic origin except red cells. Various isoforms of CD45 are generated by alternative splicing of three exons. They are all phosphotyrosine phosphatases, some of which are required for signaling through the TCR.

CD46

Also called membrane cofactor protein. CD46 is a receptor for C3b and C4b. Once bound, these complement components are destroyed by factor I. It is a 50-kDa glycoprotein expressed on T cells, B cells, monocytes, granulocytes, NK cells, platelets, fibroblasts, endothelial cells, and epithelial cells, but not on red cells.

CD48

A 47-kDa GPI-linked glycoprotein expressed on all blood lymphocytes. It is a ligand for CD2 and CD247 in rodents.

CD49

A family of 170-kDa integrin α chains that are associated with the CD29 β chain. They are expressed in various forms on leukocytes, platelets, and epithelial cells. Also called very late antigens (VLAs), their ligands are extracellular matrix proteins.

CD51

A 125-kDa integrin α chain found on platelets and endothelial cells. Its β chain is CD61, and its ligand is vitronectin.

CD54

Also called ICAM-1, this glycoprotein of 90 kDa is the ligand for the CD11a/CD18 and CD11b/CD18 integrins. It is expressed on a wide variety of cells, most notably vascular endothelial cells. It has also been reported to bind to CD43.

CD55

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Also called decay accelerating factor, this glycoprotein of 60 to 70 kDa blocks the assembly of C3 convertase and accelerates its disassembly. It thus protects normal cells against attack by complement. It is broadly distributed on many cell types.

CD56

In humans, CD56 is found on NK cells, where it appears to play an important role in NK cell-mediated cytotoxicity. In dogs, in contrast, CD56 is found exclusively on a subset ($\approx 10\%$ to 20%) of CD3⁺ T cells where its function is unknown. It is a glycoprotein of 180 kDa.

CD58

Also called LFA-3, this is a 65-kDa glycoprotein found on most cells, where it is a ligand for CD2. Sheep CD58 is expressed on red cells so that these will bind to T cells to form E-rosettes.

CD59

A glycoprotein of 18 to 20 kDa expressed on leukocytes, vascular endothelium, and epithelial cells. Also called protectin, it is the major inhibitor of the terminal complement pathway by binding to C8 and C9 and blocking the assembly of the membrane attack complex.

CD61

A $\beta 3$ integrin of 105 kDa that associates with CD41 to bind to extracellular matrix proteins. It is expressed on platelets and macrophages.

CD62

The selectins, a family of S-lectins expressed on platelet lymphocytes and endothelial cells. They bind to carbohydrate structures such as CD15s (sialyl Lewis^x) on neutrophils. CD62E is E selectin (115 kDa), CD62L is L-selectin (75 to 80 kDa), and CD62P is P-selectin (150 kDa).

CD64

Also called Fc γ RI, this high-affinity IgG receptor is a glycoprotein of 72 kDa expressed on monocytes and interferon- γ -stimulated granulocytes. It plays a key role in antibody-dependent cellular cytotoxicity.

CD66e

Also called carcinoembryonic antigen, this 180- to 200-kDa glycoprotein is expressed in large quantities by malignant intestinal cells. Its detection is therefore diagnostic for intestinal malignancy in humans.

CD71

A 95-kDa transferrin receptor expressed on activated leukocytes. It is required by dividing cells to import iron. It may also act as a selective IgA receptor.

CD72

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Found on B cells (but not plasma cells), CD72 is a 42-kDa glycoprotein ligand for CD5. It may participate in an alternative pathway of B cell and T cell activation.

CD74

The γ or invariant chain of 32 kDa associated with intracellular MHC class II molecules. Found in all MHC class II positive cells. It is believed to prevent the premature binding of endogenous peptides.

CD79

CD79a is an alternative name for the BCR signal-transducing peptide, Ig- α , and CD79b is another name for Ig- β . Both are glycoproteins of 33 to 40 kDa.

CD80

Also called B7-1, this is a 60-kDa member of the immunoglobulin superfamily expressed on a subset of antigen-presenting B cells and macrophages. It is a high-affinity receptor for CD28 and CD152 (CTLA-4). 529

The interaction of CD80 with its ligands is crucial to T cell communication with antigen-presenting cells. 530

CD81

Also called TAPA-1, CD81 is a widely expressed cell surface protein that regulates both B and T cell responses. On B cells it forms a complex with CD19, CD21, and Leu13 and is involved with co-stimulation of T cells.

CD83

A member of the immunoglobulin superfamily and a marker of mature dendritic cells. Its function is unknown.

CD85

A family of leukocyte Ig-like receptors (LIRs) (or Ig-like transcripts, ILTs) expressed on macrophages, dendritic cells, and B cells. They act as receptors for MHC class I molecules.

CD86

Related to CD80 and also called B7-2, this is a 60-kDa glycoprotein expressed on antigen-presenting macrophages, activated B cells, and dendritic cells. Its ligands are CD28 and CD152 (CTLA-4). Like CD 80 this receptor is critical for the interaction between T cells and antigen-presenting cells.

CD88

The C5a receptor found on granulocytes, macrophages, and mast cells. It is a 42-kDa glycoprotein.

CD89

This IgA receptor (Fc α R) is a 50- to 70-kDa glycoprotein expressed on granulocytes, monocytes, and some subpopulations of T and B cells.

CD90

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Otherwise known as Thy-1, this glycoprotein of 25 to 35 kDa is expressed on thymocytes and T cells in some species. It is also expressed on some brain cells.

CD91

The heat-shock protein receptor. This protein, expressed on macrophages, is important in the intracellular processing of these molecules.

CD93

The C1q receptor. Found on monocytes and neutrophils but not lymphocytes. It modulates phagocytosis of apoptotic cells.

CD94

One of the NK cell receptors. This is a C-type lectin that binds target cell MHC class I molecules.

CD95

Otherwise known as fas, this is a receptor for fas-ligand (CD95L or CD178) and a signaling component of an important cell death pathway. It is a 42-kDa glycoprotein found on myeloid and T cells. It plays a key role in the negative selection of self-reactive T cells.

CD102

Also called ICAM-2, a glycoprotein of 60 kDa expressed on vascular endothelial cells, resting lymphocytes, and monocytes but not neutrophils. It is the ligand for the integrin CD11a/CD18.

CD105

The TGF- β receptor, a glycoprotein of 95 kDa expressed on endothelial cells.

CD106

Also called VCAM-1, a glycoprotein of 100 to 110 kDa expressed on endothelial cells. It is the ligand for CD49d/CD29 (VLA-4).

CD115

The M-CSF receptor is a 150-kDa glycoprotein expressed on macrophages and their precursors.

CD116

The α chain of the GM-CSF receptor is a 43-kDa glycoprotein found on granulocytes, monocytes, and eosinophils. It shares a common β chain with IL-3R and IL-5R.

CD117

Also called c-kit, this is the receptor for stem cell factor. It is an immunoglobulin superfamily tyrosine kinase of 145 kDa found on hematopoietic precursor cells.

CDw118

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The receptor for IFN- α and IFN- β .

CD119

The IFN- γ receptor is a 90-kDa glycoprotein found on B cells, macrophages and monocytes, fibroblasts, and endothelial cells.

CD120

There are two TNF receptors (TNFR-I [CD120a] and TNFR-II [CD120b]). These are glycoproteins of 55 and 75 kDa, respectively, expressed on most cells. TNFR-I is found at higher levels on epithelial cells, whereas TNFR-II is more highly expressed on myeloid cells.

CD121

These are the two IL-1 receptors, IL-1RI (80-kDa) and IL-1RII (60- to 70-kDa) glycoproteins expressed on thymocytes, fibroblasts, keratinocytes, endothelial cells (type I) and macrophages and B cells (type II).

CD122

The IL-2R β chain. This 75-kDa glycoprotein is expressed on T cells, activated B cells, NK cells, and monocytes.

CD123

The IL-3 receptor α chain.

CD124

The IL-4 receptor, a glycoprotein of 87 kDa expressed on T and B cells, fibroblasts, endothelial cells, and stem cells.

CD125

The IL-5 receptor α chain.

CD126

The α chain of the IL-6 receptor. An 80-kDa glycoprotein expressed on B cells, plasma cells, epithelial cells, and hepatocytes.

CD127

The IL-7 receptor is a glycoprotein of 75 kDa expressed on stem cells, T cells, and monocytes.

CDw128

The IL-8 receptors are 58- to 67-kDa glycoproteins expressed on leukocytes and keratinocytes. Also called CXCR1 and 2.

CD130

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The β chain of the IL-6 (with CD126) and IL-11 receptors is a 130-kDa glycoprotein found mainly on B cells but expressed at lower levels on most leukocytes, epithelial cells, hepatocytes, and fibroblasts.

CD131

The common β chain of the IL-3 (with CD123), IL-5 (with CD125), and GM-CSF (with CD123) receptors.

CD132

The common γ chain of IL-2 (with CD25 and CD122), IL-4 (with CD124), IL-7 (with CD127), IL-9 (with CD129), and IL-15 receptors.

CD134

A member of the TNF receptor family that serves as the cell-binding receptor of feline immunodeficiency virus.

CD140

Platelet-derived growth factor (PDGF) receptor.

CD152

Also known as CTLA-4, this is the ligand for CD80 and CD86 and a negative regulator of T cell activation.

CD154

A 35-kDa member of the TNF family. Since it serves as the ligand for CD40, it is also called CD40L. Found on activated Th cells, it plays a key role in T cell activation by cross-linking with CD40 on antigen presenting cells.

CD158

The KIR family of MHC class I receptors expressed on NK cells and T cell subsets. They play a key role in NK cell activation.

CD166

An activated leukocyte adhesion molecule that acts as a receptor for IL-6.

CD169

Siglec-1 or sialoadhesin, a macrophage lectin-like adhesion molecule.

CD172a

A signal regulatory protein expressed on monocytes and a subset of dendritic cells.

CD178

Fas-ligand (CD95-L). A member of the tumor necrosis factor superfamily, this is a key molecule in the induction of cell death by apoptosis.

CD181-CD185

CXCR1-CXCR5 chemokine receptors.

CD191-D199

CCR1-CCR9 chemokine receptors.

CD206

A type I membrane protein found on mature macrophages and immature dendritic cells. It is a C-type lectin that recognizes certain carbohydrate ligands such as those rich in mannose.

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CD209

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DC-SIGN found on a subset of dendritic cells.

CDw210

IL-10R cytokine receptor.

CD212

IL-12R β chain.

CD213

IL-13R α chain (and a member of the IL-4 receptor complex).

CD217

IL-17R cytokine receptor.

CD218a and b

IL-18R cytokine receptor α and β chains.

CD220

Insulin receptor.

CD221

IGF1R cytokine receptor.

CD230

The prion protein (PrP). A large membrane protein found on neurons. The abnormal form of this protein (PrP^{Sc}) is a transmissible agent causing spongiform encephalopathies.

CD233

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An erythrocyte membrane protein that functions as an anion (chloride and bicarbonate) exchanger. Also called band 3 protein (see [Chapter 31](#)).

CD240

Rhesus blood group molecules.

CD247

The T cell antigen receptor zeta (ζ) chain.

CD281-CD290

TLR1 through TLR10.

CD295

The leptin receptor.

CD314

NKG2D, the receptor for MICA and MICB.

CD327-CD329

Siglecs 6, 7, and 9.

CD331-CD334

FGF receptors 1 through 4.

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39.2 APPENDIX 2 Some Selected Cytokines

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Granulocyte colony-stimulating factor (G-CSF)

A glycoprotein of 20 kDa produced by macrophages, endothelial cells, and fibroblasts. It regulates the maturation of granulocyte progenitors into mature neutrophils. The term colony-stimulating factor refers to its ability to promote the growth of bone marrow stem cell “colonies” in tissue culture.

Granulocyte-macrophage colony-stimulating factor (GM-CSF)

A protein of 14 kDa produced by T cells, macrophages, fibroblasts, and endothelial cells. It is the major regulator of granulocyte and macrophage stem cells. It induces phagocytosis, superoxide production, and ADCC by neutrophils.

High-mobility group box protein-1 (HMGB1)

A chromatin-binding protein of 28 kDa that is either actively secreted by inflammatory cells such as macrophages, or passively released from necrotic cells. HMGB1 acts through TLRs to promote macrophage cytokine release, thus enhancing inflammation. It is an attractant for vascular smooth muscle cells. It has bactericidal activity and is a potent inducer of fevers.

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Interferon- α (IFN- α)

Produced in at least 23 different variants, with molecular masses ranging from 19 to 26 kDa. It has a common conserved sequence region but highly variable amino terminus. It is produced in large quantities by plasmacytic dendritic cells and in much smaller amounts by lymphocytes, monocytes, and macrophages. It activates NK cell-mediated cytotoxic activity, and it stimulates the differentiation of monocytes into dendritic cells, as well as the maturation and activity of dendritic cells. IFN- α also drives certain γ/δ T cell responses. It has, of course, potent antiviral activities.

Interferon- β (IFN- β)

A 20-kDa protein produced by fibroblasts and is coded for by a single gene in most mammals. Produced in response to viral infections, it has similar properties to IFN- α .

Interferon- γ (IFN- γ)

The only type II interferon, it is a 17-kDa glycoprotein produced mainly by CD4⁺ Th1 cells, by some CD8⁺ T cells, and by NK cells. IFN- γ acts on B cells, T cells, NK cells, and macrophages and is the key mediator of cell-mediated immune responses.

Interferon- ω (IFN- ω)

A protein of 20 kDa produced by lymphocytes and monocytes and human, horse, pig, rabbit, and dog trophoblast cells. It has significant antiviral activity.

Interferon- τ (IFN- τ)

A protein of 20 kDa produced by ruminant trophoblast cells during early pregnancy. It regulates immune responses in the placenta.

Interferon- δ (IFN- δ)

A protein of 19 kDa produced by pig trophoblast cells. It probably controls the maternal immune response to the fetus.

Interferon- λ (IFN- λ)

A collective name for IL-28A, IL-28B, and IL-29. All are 20-kDa proteins distantly related to the IL-10 family. All three use a distinct receptor system to trigger antiviral defense.

Interleukin-1 (IL-1)

A family of at least 11 proteins produced by macrophages, dendritic cells, T cells, B cells, NK cells, vascular endothelium, fibroblasts, and keratinocytes. They originate as 31-kDa procytokines but are cleaved by caspase-1 to 17-kDa active peptides. The two most important forms of IL-1 act on Th2 cells, B cells, NK cells, neutrophils, eosinophils, dendritic cells, fibroblasts, endothelial cells, and hepatocytes. It is a proinflammatory mediator and a Th2 cell stimulator.

Interleukin-2 (IL-2)

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A 15.4-kDa glycoprotein produced by Th1 and NK cells. Its targets include other T cells, B cells, and NK cells. IL-2 activates helper and cytotoxic T cells and NK cells. IL-2 stimulates T cell proliferation and cytotoxicity.

Interleukin-3 (IL-3)

A 15-kDa protein produced by activated T cells, NK cells, eosinophils, and mast cells. It is a hematopoietic growth factor that stimulates the growth and maturation of bone marrow stem cells for eosinophils, neutrophils, and monocytes.

Interleukin-4 (IL-4)

A 15-kDa protein produced by activated Th2 cells, mast cells, and activated basophils. It acts on B cells, T cells, macrophages, endothelial cells, fibroblasts, and mast cells. IL-4 stimulates the growth and differentiation of B cells.

Interleukin-5 (IL-5)

A 26-kDa disulfide-linked homodimeric glycoprotein produced by activated Th2 cells, mast cells, and eosinophils. In humans its main activity is the control of eosinophil production.

Interleukin-6 (IL-6)

A 20- to 30-kDa glycoprotein that occurs in at least 5 isoforms. It is produced by activated macrophages, T and B cells, mast cells, vascular endothelial cells, fibroblasts, keratinocytes, and mesangial cells. It is also produced by muscle cells during exercise. It acts on T cells, B cells, hepatocytes, and bone marrow stromal cells as well as the brain.

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Interleukin-7 (IL-7)

A 17-kDa glycoprotein produced by bone marrow and thymic stromal cells. It regulates the activity of lymphoid stem cells. Its major role, however, is to control lymphocyte function by regulating V(D)J recombination in both B and T cells.

Interleukin-8 (IL-8)

The prototypical chemokine (CXCL8). Like other chemokines, it is a relatively small (8.4-kDa) protein produced by macrophages and endothelial cells. IL-8 attracts and activates neutrophils.

Interleukin-9 (IL-9)

A 14-kDa stem cell growth factor produced by Th2 cells. It promotes the growth of helper T cells and mast cells. It also potentiates the effects of IL-4 on IgE production.

Interleukin-10 (IL-10)

An 18.6-kDa nonglycosylated homodimeric protein that acts as an immunosuppressive and antiinflammatory cytokine that suppresses inflammation as well as T cell, NK cell, and macrophage function. It is mainly produced by Th2 cells but also from M2 cells, NK cells, and some dendritic cells. Its targets are Th1 cells, B cells, macrophages, NK cells, and mast cells.

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Interleukin-11 (IL-11)

A 19-kDa nonglycosylated protein produced by bone marrow stromal cells, epithelial cells, and fibroblasts. It stimulates B cell growth in association with IL-6. IL-11 also stimulates megakaryocyte formation in association with IL-3 and promotes the production of acute-phase proteins.

Interleukin-12 (IL-12)

A 75-kDa heterodimer consisting of disulfide-linked 35- and 40-kDa subunits (p35 and p40). It is produced by monocytes and macrophages, dendritic cells, B cells, and keratinocytes. It is the major activator of Th1 cells and NK cells.

Interleukin-13 (IL-13)

A 12.5-kDa glycoprotein produced by Th2 cells, cytotoxic T cells, mast cells, and dendritic cells. It has biological activities similar to those of IL-4, because it acts through a receptor (CD213) that shares a common α chain with the IL-4R.

Interleukin-14 (IL-14)

A 53-kDa glycoprotein produced by T cells and some malignant B cells. It is a B cell growth factor that inhibits immunoglobulin secretion and selectively expands some B cell subpopulations.

Interleukin-15 (IL-15)

A 14-kDa glycoprotein produced by activated macrophages, dendritic cells, endothelial cells, and fibroblasts. It shares many biological activities with IL-2. IL-15 acts as a T cell, B cell, and NK cell growth factor. IL-15 is essential for the prolonged survival of memory T cells.

Interleukin-16 (IL-16)

A 13-kDa protein produced by CD8⁺ T cells, eosinophils, dendritic cells, and mast cells. Its receptor is CD4 through which IL-16 regulates CD4⁺ T cell recruitment and activation. It also acts on eosinophils and macrophages.

Interleukin-17 (IL-17)

A mixture of at least 6 proteins produced mainly by Th17 helper cells. They exist as 31- to 40-kDa disulfide-linked homodimers. IL-17 molecules stimulate macrophages and endothelial cells to secrete proinflammatory cytokines and chemokines leading to the recruitment and activation of neutrophils. IL-17 thus appears to play a key role in the development of acute inflammation.

Interleukin-18 (IL-18)

A member of the IL-1 family that is produced, like IL-1, by antigen-presenting cells. It originates as a 24-kDa pro-protein that is cleaved by caspase-1 to an 18-kDa active molecule. It activates Th1 cells to promote the production of IFN- γ , TNF- α , IL-1, CD95L, and several chemokines. This can lead to positive feedback where the IL-18 and IFN- γ reinforce each other's activities.

Interleukin-19 (IL-19)

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An 18-kDa protein and a member of the IL-10 family produced by B cells and activated monocytes. It is a proinflammatory cytokine that acts on monocytes to stimulate production of IL-1, IL-6, and TNF- α .

Interleukin-20 (IL-20)

A 35.2-kDa homodimeric protein and a member of the IL-10 family. It is produced by monocytes and keratinocytes and acts as a hematopoietic growth factor.

Interleukin-21 (IL-21)

A protein of 15-kDa produced by activated Th2 cells and structurally related to IL-2 and IL-15. It regulates NK, B, and T cell function. It upregulates production of IL-18R and IFN- γ .

Interleukin-22 (IL-22)

A 33.6-kDa homodimeric protein and a member of the IL-10 family produced by activated Th17 cells and mast cells. It induces acute-phase protein production in the liver. IL-22 acts on cells of the skin and digestive and respiratory systems to increase expression of several b-defensins and presumably promotes innate immunity in these tissues.

Interleukin-23 (IL-23)

A heterodimer consisting of a 19kDa peptide chain (IL-23p19) paired with the IL-12p40 chain. IL-23 is produced by macrophages, dendritic cells, and activated γ/δ T cells. It is a major cytokine produced by activated M1 cells. It stimulates Th17 cells to secrete IL-17 and IL-22, and these T cells in turn promote acute neutrophil-mediated inflammation.

Interleukin-24 (IL-24)

A 37-kDa dimeric protein is a member of the IL-10 family produced by activated monocytes and Th2 cells. It is involved in antitumor activity since it stimulates apoptosis in many tumor cell lines and stimulates acute-phase responses in hepatocytes. It may play a role in wound healing.

Interleukin-25 (IL-25)

Unassigned.

Interleukin-26 (IL-26)

A 37-kDa protein is a member of the IL-10 family produced by virus-transformed CD4⁺T cells, activated memory cells, and activated T and NK cells. It induces the proliferation of keratinocytes and T cells. It signals through a novel receptor composed of IL-20 receptor 1 and IL-10 receptor 2.

Interleukin-27 (IL-27)

A heterodimer with one chain (p28) also called IL-30 and the other called EB-13 related to IL-12p40. It is expressed by activated monocytes and dendritic cells. IL-27 suppresses the activation of all three helper T cell subsets and prevents neutrophil activation. It thus serves a major regulatory role.

Interleukin-28 (IL-28)

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Two protein isoforms of 22 kDa produced by virus-infected cells. IFN-2 (IL-28A) and IFN-3 (IL-28B) share a common three-dimensional structure with IL-10 but have limited sequence similarity.

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Interleukin-29 (IL-29) (IFN-11)

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A 20-kDa member of the IL-10 family produced by virus-infected cells. It is an interferon closely related to IL-28A and IL-28B.

Interleukin-30 (IL-30)

A 28-kDa protein secreted by antigen-presenting cells. It forms one chain of heterodimeric IL-27. It acts on naive CD4 T cells. It synergizes strongly with IL-12 to promote IFN- γ production by Th1 cells.

Interleukin-31 (IL-31)

A 15.5-kDa protein related to IL-6. It is produced by stimulated Th2 cells. Its receptor is expressed on keratinocytes and induced on monocytes by IFN- γ . It may be involved in the pathogenesis of allergic skin diseases.

Interleukin-32 (IL-32)

A protein of 15 kDa that exists in four isoforms. It is produced by activated human lymphocytes, NK cells, and endothelial cells. It acts on macrophages to enhance the production of TNF- α , IL-1 β , IL-6, and IL-8.

Interleukin-33 (IL-33)

An 18-kDa member of the IL-1 family that serves as a matched counterpart to IL-18. Like IL-1, it is derived from cleavage of a 31-kDa precursor by caspase-1. IL-33 is expressed by endothelial and smooth muscle cells. It is a chromatin-associated nuclear factor. It acts through an IL-1 receptor to drive production of IL-4, IL-5, and IL-13 by Th2 cells. IL-33 may play a key role in Th2 diseases.

Leptin

A 16-kDa globular protein produced by adipocytes. This cytokine suppresses appetite by signaling through a receptor in the hypothalamus.

Macrophage *colony-stimulating factor* (M-CSF)

An 80- to 100-kDa glycoprotein. Its active form is a disulfide-linked dimer. It is a hematopoietic factor produced by lymphocytes, macrophages, fibroblasts, epithelial cells, and endothelial cells. These act on monocyte stem cells to induce their proliferation and differentiation and promote macrophage cytotoxicity.

Transforming growth factor- β (TGF- β)

Belongs to a family of at least 45 signaling proteins. It is a 25-kDa protein consisting of disulfide-linked homodimers. There are three isoforms of this protein that act through the same receptor and have identical biological properties. They are produced by platelets, activated macrophages, neutrophils, B cells, and T cells and act on most cell types, including T and B cells, dendritic cells, macrophages, neutrophils, and fibroblasts. The TGF- β s regulate cell division, enhance the deposition of extracellular matrix proteins, and are immunosuppressive.

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Tumor necrosis factor- α (TNF- α)

A 17-kDa protein produced by macrophages, mast cells, T cells, endothelial cells, B cells, adipocytes, and fibroblasts. It forms a noncovalently linked trimer in solution. TNF- α is the central inducer of inflammation.

Tumor necrosis factor- β (TNF- β)

A 19-kDa protein produced by Th1 cells and activated CD8⁺ T cells. It is either secreted in a soluble form or forms a complex with lymphotoxin- β in the T cell membrane. TNF- β kills tumor cells and activates neutrophils, macrophages, endothelial cells, and B cells.

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Glossary

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Activated macrophage

A macrophage in a state of enhanced metabolic and functional activity.

Active immunity

Immunity produced as a result of administration of an antigen, thus triggering an immune response.

Acute inflammation

Rapidly developing inflammation of recent onset. It is characterized by tissue infiltration by neutrophils.

Acute phase proteins

Proteins, synthesized by the liver, whose level in serum rises rapidly in response to acute inflammation and tissue damage.

Adjuvant

Any substance that, when given with an antigen, enhances the immune response to that antigen.

Adoptive immunity

Immunity that results from the transfer of cells from an immunized animal to an unimmunized recipient.

Affinity

The strength of binding between two molecules such as an antigen and antibody. Usually expressed as an association constant (K_a).

Affinity maturation

The progressive increase in antibody affinity for antigen that occurs during the course of an immune response as a result of somatic mutation in V genes.

Agammaglobulinemia

The absence of gamma globulins in blood.

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Agglutination

The clumping of particulate antigens by antibody.

Agnatha

A class of jawless fish. It includes the cyclostomes, an order containing the hagfish and lamprey.

Alarmins

Molecules released by dead or damaged tissues that trigger innate immune responses, especially inflammation.

Albumin

The major serum protein of 60 kDa.

Alleles

Different forms of a gene that occupy the same polymorphic locus.

Allelic exclusion

The expression of only one allelic protein by a cell from a heterozygous individual that has the genes to express both allelic proteins.

Allergens

Antigens that provoke allergic reactions. Usually type I hypersensitivity.

Allergic contact dermatitis

An inflammatory skin reaction mediated by Th1 cells responding to low-molecular-weight chemicals bound to skin cells.

Allergy

A hypersensitivity reaction initiated by specific immunological mechanisms.

Allogeneic

Genetically dissimilar animals of the same species.

Allograft

An organ graft between two genetically dissimilar animals of the same species.

Allotype

Antigenic (and structural) differences between the proteins of different individuals of the same species as a result of transcription of different alleles.

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Alternative complement pathway

The complement pathway triggered by the activation of C3 by the presence of an activating surface.

Amyloid

An extracellular, amorphous, waxy protein deposited in the tissues of individuals with a chronic inflammation or a myeloma.

Analog

An organ or tissue that has the same function as another but is of different evolutionary origin.

Anamnestic response

A secondary immune response.

Anaphylatoxins

Complement fragments that stimulate mast cell degranulation and smooth muscle contraction.

Anaphylaxis

A severe, life-threatening generalized or systemic hypersensitivity reaction.

Anergy

The failure of a sensitized animal to respond to an antigen—a form of immunological tolerance.

Antibiotic

A chemical compound, usually obtained from microorganisms, that can prevent growth or kill bacteria. Do not confuse this with antibody.

Antibody

An immunoglobulin molecule synthesized on exposure to antigen, which can combine specifically with that antigen.

Antibody-dependent cellular cytotoxicity

The killing of antibody-coated target cells by cytotoxic cells with surface Fc receptors.

Antigen

Any foreign substance that can bind to specific lymphocyte receptors and so induce an immune response.

Antigen-presenting cells

Cells that can ingest, process, and present antigen to antigen-sensitive cells in association with MHC class I and class II molecules.

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Antigen processing

The series of events that modify antigens so that they bind to MHC molecules and so can be recognized by antigen-sensitive cells.

Antigen-sensitive cells

Cells that can bind and respond to specific antigen.

Antigenic determinant

See Epitope.

Antigenic variation

The progressive change in surface antigens exhibited by viruses, parasites, and some bacteria in order to evade destruction.

Antigenicity

The ability of a molecule to be recognized by an antibody or lymphocyte.

Antiglobulin

Antibody made against an immunoglobulin, usually by injecting immunoglobulin into an animal of another species.

Antiglobulin test

A technique for detecting the presence of nonagglutinating antibody on the surface of a particle.

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Antiserum

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Serum that contains specific antibodies. Synonymous with immune globulin.

Antitoxin

Antiserum directed against a toxin and used for passive immunization.

Anurans

An order of advanced amphibians that includes the frogs and toads.

Apoptosis

The controlled self-destruction of a cell; one form of programmed cell death. (Apoptosis is a Greek word describing the falling away of petals from flowers or the leaves from trees.)

Arthus reaction

Local inflammation due to a type III hypersensitivity reaction; it is induced by the injection of antigen into the skin of an immunized animal.

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Asthma

A type I hypersensitivity disease characterized by a reduction in airway diameter, leading to difficulty in breathing (dyspnea).

Atopy

A genetic predisposition to become sensitized and produce IgE antibodies in response to allergens commonly occurring in the environment.

Attenuation

The reduction of virulence of an infectious agent.

Autoantibodies

Antibodies directed against antigens on normal body tissues.

Autoantigen

A normal body component that acts as an antigen.

Autograft

A tissue or organ graft made between two sites within the same animal.

Autoimmune disease

Disease caused by an immune attack against an individual's own tissues.

Autoimmunity

The process of mounting an immune response against a normal body component.

B lymphocytes (B cells)

Lymphocytes that have undergone a period of processing in the bursa or its mammalian equivalent. They are responsible for antibody production.

Bacille Calmette-Guérin vaccine

An attenuated strain of *Mycobacterium bovis*. This may be used as a specific vaccine or as a nonspecific immune stimulator.

Bacterin

A preparation of killed bacteria used for immunization.

Basophil

A polymorphonuclear cell that contains granules with a high avidity for basic dyes such as hematoxylin. It participates in type I hypersensitivity reactions.

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BCG

See Bacille Calmette-Guérin Vaccine.

Bence-Jones protein

Immunoglobulin light chains found in the urine of patients with myelomas. They precipitate out of solution when the urine is warmed and redissolve at higher temperatures.

Benign tumor

A tumor that does not spread from its site of origin.

Blast cells

Cells before division, when they have large amounts of cytoplasm.

Blastogenesis

The stimulation of cell division.

Blocking antibody

A noncytotoxic, noncomplement activating antibody that, by coating cells, can protect them against immune destruction.

Blood groups

Antigens found on the surface of red blood cells. Their expression is inherited.

Bursectomy

Surgical removal of the bursa of Fabricius.

C3 convertases

Enzymes that can cleave native C3 into C3a and C3b fragments.

Capping

The clumping of surface structures such as antigens or receptors in a small area on the surface of a cell.

Capsid

The protein coat around a virus.

Carcinoma

A tumor originating from cells of epithelial origin.

Carrier

An immunogenic macromolecule to which a hapten may be bound, thus making the hapten immunogenic.

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Cascade reactions

A linked series of enzyme reactions in which the products of one reaction catalyze a second reaction, and so forth.

CD molecule

A cell surface molecule classified according to the internationally accepted CD system. CD numbers are assigned to cell surface molecules based on their reactivity with a panel of monoclonal antibodies.

Cell-mediated cytotoxicity

The killing of target cells induced by contact with cytotoxic T cells, NK cells, or macrophages.

Cell-mediated immunity

A form of immune response mediated by T lymphocytes and macrophages; it can be conferred on an animal by adoptive transfer.

Cestodes

Parasitic tapeworms.

Chemokine

A family of proinflammatory and chemotactic cytokines with a characteristic sequence of four cysteine residues. They regulate the emigration of leukocytes from blood into tissues.

Chemotaxis

The directed movement of cells under the influence of a chemical concentration gradient.

Chimera

An animal that contains cells from two or more genetically different individuals.

Chondrichthyes

The class that contains the cartilaginous fishes, including sharks, skates, and rays.

Chromosome translocation

A form of mutation in which portions of two chromosomes switch position.

Chronic inflammation

Slowly developing or persistent inflammation characterized by tissue infiltration with macrophages and fibroblasts.

Class

The five major forms of immunoglobulin molecules common to all members of a species (see Isotype).

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Class switch

The change in immunoglobulin class that occurs during the course of an immune response as a result of heavy chain gene rearrangement.

Classical complement pathway

The complement pathway triggered by activation of C1 by antigen-antibody complexes.

Clonal deletion

The elimination of self-reactive T cells in the thymus.

Clonal selection

A key concept in immunology. The proliferation of specific lymphocyte clones in response to a specific epitope. The response is triggered through specific antigen-binding receptors.

Clone

The progeny of a single cell.

Clonotype

A clone of B cells with the ability to bind a single epitope.

Cluster of differentiation

The set of monoclonal antibodies that recognize a single protein on a cell surface. A CD antigen is by extension, therefore, a defined protein on the surface of a cell.

Coelomocyte

A phagocytic cell found in the coelomic cavity of invertebrates.

Collectins

A family of carbohydrate-binding lectins that depend on calcium for their adhesion.

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Colostrum

The secretion that accumulates in the mammary gland in the last weeks of pregnancy. It is very rich in immunoglobulins.

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Combined immunodeficiency

A deficiency in both the T cell- and B cell-mediated components of the immune system.

Complement

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A group of serum and cell-surface proteins activated by factors such as the combination of antigen and antibody and results in the generation of enzyme cascades that have a variety of biological consequences including cell lysis and opsonization.

Complementarity-determining region

Those areas within the variable regions of antibodies and T cell antigen receptors that bind to antigen and determine the molecule's antigen binding-specificity. Synonymous with hypervariable region.

Concanavalin A (Con A)

A lectin extracted from the Jack bean that makes T cells divide.

Conglutinin

A bovine mannose-binding protein that also combines with C3b.

Constant domains

Structural domains with little sequence variability found in antibodies and TCRs.

Constant region

The portion of immunoglobulin and TCR peptide chains that consists of a relatively constant sequence of amino acids.

Contrasuppression

The suppression of suppressor cells by a population of contrasuppressor T cells. Contrasuppressor cells are distinct from helper cells.

Convertase

A protease that acts on a protein to cause its activation.

Cortex

The outer region of an organ such as the thymus or lymph node.

Corticosteroids

Steroid hormones released from the adrenal cortex that have profound effects on the immune system. Some corticosteroids may be synthetic in origin.

Co-stimulators

Molecules required to stimulate an antigen-sensitive cell simultaneously with antigen in order to initiate an effective immune response.

Cross-reaction

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The reaction of an antibody or an antigen receptor directed against a specific antigen, with a second antigen. This occurs because the two antigens possess an epitope in common.

Cutaneous basophil hypersensitivity

A form of delayed hypersensitivity reaction in skin associated with an extensive basophil infiltration.

Cytokine storm

The pathological effects induced by the massive activation of T cells and, as a result, the unregulated production of many different cytokines.

Cytokines

Secreted proteins that mediate cellular interactions and regulate cell growth and secretion. As a result, they regulate many aspects of the immune system.

Cytolysis

Destruction of cells by immune processes.

Cytotoxic cell

A cell that can injure or kill other cells.

Delayed hypersensitivity

A cell-mediated inflammatory reaction in the skin, so called because it takes 24 to 48 hours to reach maximum intensity.

Dendritic cells

Cells that possess long cytoplasmic processes. Their primary role is to function as highly effective antigen-trapping and antigen-presenting cells.

Desensitization

The prevention of allergic reactions through the use of multiple injections of allergen.

Diapedesis

The emigration of cells from intact blood vessels during inflammation.

Disseminated intravascular coagulation

Activation of the clotting cascade within the circulation.

Disulfide bonds

Bonds that form between two cysteine residues in a protein. They may be either interchain (between two peptide chains) or intrachain (joining two parts of one chain).

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Domain

Discrete structural units from which protein molecules are constructed. Their sizes and amino acid sequences are very diverse.

Dysgammaglobulinemia

The abnormal production of gamma globulins in blood.

Effector cell

A cell that is able to effect an immune response. These cells include cytotoxic T cells and natural killer cells.

Electrophoresis

The separation of the proteins in a complex mixture by subjecting them to an electrical potential.

ELISA

Enzyme-linked immunosorbent assay. An immunological test that uses enzyme-linked antiglobulins and substrate bound to an inert surface.

Endocytosis

The uptake of extracellular substances by cells.

Endogenous antigen

Foreign antigen synthesized within body cells. Examples include newly formed virus proteins.

Endosomes

Cytoplasmic vesicles formed by invagination of the outer cell membrane. They contain endocytosed substances.

Endothelium

The cells that line blood vessels and lymphatics.

Endotoxins

Lipopolysaccharide components of Gram-negative bacterial cell walls.

Enhancement

Improved survival of grafts or tumor cells induced by some antibodies.

Eosinophil

A polymorphonuclear leukocyte containing characteristic granules that stain intensely with the dye eosin.

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Eosinophilia

Increased numbers of eosinophils in the blood.

Epithelioid cells

Macrophages that accumulate around a tubercle and resemble epithelial cells in histological sections.

Epitope

A site on the surface of an antigen that is recognized by an antigen receptor. As a result, immune responses are directed against specific epitopes. Synonymous with antigenic determinant.

Erythema

Redness due to inflammation.

Eukaryotic organism

An organism characterized by cells possessing a distinct nucleus and containing both DNA and RNA.

Eutherians

The placental mammals; the order to which humans belong and a dominant life form on this planet.

Exocytosis

The export of material from a cell by the fusion of cytoplasmic vesicles with the outer cell membrane.

Exogenous antigen

A foreign antigen that originates at a source outside the body; for example, bacterial antigens.

Exon

A region within a gene that is expressed.

Exotoxins

Soluble protein toxins, usually produced by Gram-positive bacteria, that have a specific toxic effect.

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Fab fragment

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The antigen-binding fragment of a partially digested antibody. It consists of light chains and the N-terminal halves of heavy chains.

Facultative intracellular organism

An organism that can, if necessary, grow within cells.

Fc receptor

A cell-surface receptor that specifically binds antibody molecules through their Fc region.

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Fc region

That part of an immunoglobulin molecule consisting of the C-terminal halves of heavy chains. It is responsible for the biological activities of the molecule.

Fibronectin

A glycoprotein responsible for adhesion between cells. It also binds foreign material to cells and thus functions as an opsonin.

First-set reaction

The rejection of a first foreign tissue graft.

Fluorescent antibody

An antibody chemically attached to a fluorescent dye.

Framework regions

The parts of a variable region of immunoglobulins and TCRs that have a relatively constant amino-acid sequence and so form a structure on which the hypervariable, complementarity-determining regions may be constructed.

Gamma (γ) globulins

Serum proteins that migrate toward the cathode on electrophoresis. They contain most of the immunoglobulins.

Gammopathies

Abnormal increases in gamma globulin levels.

Gel diffusion

An immunoprecipitation technique that involves letting antigen and antibody meet and precipitate in a clear gel such as agar.

Gene complex

A cluster of related genes occupying a restricted area of a chromosome.

Gene conversion

The exchange of blocks of DNA between different genes.

Gene segment

Another term for exon. It tends to be used exclusively to denote the exons that code for immunoglobulin and TCR V, D, and J regions.

Genes

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Units of DNA that code for the amino acid sequence of a protein.

Germinal center

A structure characteristic of many lymphoid organs, in which rapidly dividing B cells form a pale-staining spherical mass surrounded by a zone of dark-staining cells. This is the location where somatic mutation occurs and memory cells are generated.

Globulins

Serum proteins precipitated by the presence of a half-saturated solution of ammonium sulfate.

Glomerulonephritis

Pathological lesions in the glomeruli of the kidney.

Glycoform

Differing molecular forms of a protein resulting from differences in glycosylation.

Glycoprotein

A protein that contains carbohydrate.

G-proteins

GTP-binding proteins that act as signal transducers for many cell surface receptors.

Graft-versus-host disease

Disease caused by an attack of transplanted lymphocytes (usually in the form of a bone marrow allograft) on the cells of a histoincompatible and immunodeficient recipient.

Granulocyte

A myeloid cell containing prominent cytoplasmic granules. They include neutrophils, eosinophils, and basophils.

Granuloma

An inflammatory lesion characterized by chronic inflammation with mononuclear cell infiltration and extensive fibrosis.

Granzyme

A family of proteases found in the granules of cytotoxic T cells.

Growth factors

Molecules that promote cell growth.

Haplotype

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The complete set of linked alleles within a gene complex. They are inherited as a group and determine a specific phenotype.

Hapten

A small molecule that cannot initiate an immune response unless first bound to an immunogenic carrier molecule.

Heat-shock proteins

Proteins synthesized by cells in response to many different physiological stresses. Their function is to act as chaperones and carry proteins within different subcompartments of a cell.

Helminths

Worms, many of which are parasites and so stimulate immune responses.

Helper T cells

The subpopulation of T cells that promote immune responses by providing co-stimulation from cytokines and co-stimulatory receptors.

Hemagglutination

The agglutination of red blood cells.

Hematopoietic organ

An organ in which blood cells are produced.

Hemocytes

Phagocytic cells found in invertebrate hemo-lymph.

Hemolymph

The fluid that fills the body cavities of invertebrates. It has functions analogous to those of blood.

Hemolysin

An antibody that can lyse red blood cells in the presence of complement.

Hemolytic disease

Disease occurring as a result of the destruction of red blood cells by antibodies transferred to the young animal from its mother.

Herd immunity

Immunity conferred on a population as a result of the presence of immune individuals within that population.

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Heterodimer

A molecule consisting of two different subunits.

Heterophile antibodies

Antibodies that react with epitopes found on a wide variety of unrelated molecules.

High endothelial venule

A specialized blood vessel lined with high epithelium, found in the paracortex of lymph nodes and other lymphoid organs.

Hinge region

The region between the first and second constant domains in some immunoglobulin molecules that permits them to bend freely.

Histiocytes

Tissue macrophages.

Histocompatibility molecules

Cell membrane proteins that are required to present antigen to antigen-sensitive cells.

Homodimer

A molecule consisting of two identical subunits.

Homolog

A part similar in structure, position, and origin to another organ.

Homology

The degree of sequence similarity between two genes (nucleotide sequences) or two proteins (amino acid sequences).

Humoral immunity

An immune response mediated by antibodies.

Hybridoma

A cell line formed by the fusion of a myeloma cell with a normal antibody-producing cell.

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Hypersensitivity

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Reproducible clinical signs initiated by exposure to an antigen at a dose tolerated by normal individuals.

Hypersensitivity pneumonitis

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Inflammation in the lung caused by a type III hypersensitivity reaction to inhaled antigen within the alveoli.

Hypervariable regions

Areas within immunoglobulin or TCR variable regions where the greatest variations in amino acid sequence occur and which therefore bind antigens.

Hypogammaglobulinemia

Low levels of gamma globulins in blood.

Idiotope

An epitope located in the variable region of an immunoglobulin molecule.

Idiotypic

The collection of idiotopes on an immunoglobulin molecule.

Idiotypic networks

The series of reactions among idiotypes, anti-idiotypes, and anti-anti-idiotypes that play a role in controlling immune responses.

Immediate hypersensitivity

The hypersensitivity reaction mediated by IgE and mast cells. Otherwise known as type I hypersensitivity.

Immune complex

Another term for antigen-antibody complexes.

Immune elimination

The removal of an antigen from the body by circulating antibodies and phagocytic cells.

Immune exclusion

The prevention of absorption of antigens from body surfaces by immunoglobulin A.

Immune globulin

An antibody preparation containing specific antibodies against a pathogen and used for passive immunization.

Immune paralysis

Tolerance induced by very high doses of antigen.

Immune response genes

MHC class II genes, so called because they regulate the ability of an animal to respond to specific antigens.

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Immune stimulants

Compounds, commonly bacterial in origin, that stimulate the immune system by promoting cytokine release from macrophages.

Immune surveillance

The concept that lymphocytes survey the body for cancerous or abnormal cells and then eliminate them.

Immunity

The state of resistance to an infection.

Immunization

The administration of an antigen to an individual in order to confer immunity.

Immunoconglutinins

Autoantibodies directed against activated complement components.

Immunodeficiency

Diseases in which immune function is partially or totally deficient.

Immunodiffusion

Another name for the gel diffusion technique.

Immunodominant

The epitope on a molecule that provokes the most intense immune response.

Immunoelectrophoresis

A procedure involving electrophoresis in gel followed by immunoprecipitation; it is used to identify the proteins in a complex solution such as serum.

Immunofluorescence

Immunological tests that make use of antibodies conjugated to a fluorescent dye.

Immunogenetics

That portion of immunology that deals with the direct effects of genes on the immune system.

Immunogenicity

The ability of a molecule to elicit an immune response.

Immunoglobulin

A glycoprotein with antibody activity.

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Immunoglobulin superfamily

A family of proteins that contain characteristic immunoglobulin domains.

Immunological paralysis

A form of immunological tolerance in which an ongoing immune response is inhibited by the presence of large amounts of antigen.

Immunological synapse

The area of contact between an antigen-presenting cell and a lymphocyte such as a T or B cell. Within the synapse, cell-surface molecules are arranged in a well-defined pattern designed to optimize signaling between the cells.

Immunoperoxidase

Immunological test that makes use of antibodies chemically conjugated to the enzyme peroxidase.

Immunosuppression

Inhibition of the immune system by drugs or other processes.

Inactivated vaccine

A vaccine containing an agent that has been treated in such a way that it can no longer replicate in the host.

Incomplete antibody

An antibody that can bind to a particulate antigen but cannot make it agglutinate.

Indurated

Hardened.

Inflammation

The responses of tissues to injury. These responses enhance tissue defenses and initiate repair.

Inflammatory macrophage

A partially activated macrophage associated with microbial invasion, tissue damage, or inflammation.

Innate immunity

Immunity present in all animals that need not be induced by prior exposure to an infectious agent. It is mediated by proteins encoded in the germline.

Inoculation

The administration of a vaccine by injection or scratching.

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Integral membrane protein

Cell surface proteins that are integral components of the cell membrane as opposed to proteins that are passively adsorbed to cell surfaces.

Integrins

A family of adhesion proteins found on cell membranes that bind either to ligands on the surface of other cells or to connective tissue proteins such as fibronectin or collagen.

Interchain bond

A bond between two different peptide chains. Usually formed by a disulfide linkage between two cysteine residues.

Interdigitating cell

A form of dendritic cell found within lymphoid organs.

Interferons

Cytokines that can interfere with viral replication. Some interferons play an important role in the regulation of immunity.

Interleukins

Proteins that act as growth and differentiation factors for the cells of the immune system.

Intrachain bond

A bond between two cysteine residues on a single peptide chain. Because disulfide bonds are short, its effect is to produce a fold in the peptide chain.

Intraepithelial lymphocytes

Lymphocytes, mainly T cells, located among the epithelial cells in the intestinal wall.

Intron

A region within a gene that separates exons and is not expressed.

Isoform

Different molecular forms of a protein that are generated by differential processing of RNA transcripts of a single gene.

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Isogeneic (syngeneic)

Genetically identical.

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Isograft

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A graft between two genetically identical animals.

Isotype

These are closely related proteins that arise as a result of gene duplication. They are found in all animals of a species. Thus the classes and subclasses of immunoglobulins are actually isotypes.

Isotype switching

The change in immunoglobulin class that occurs during the course of the immune response as a result of heavy chain gene switching.

J chain

A short peptide that joins units in the polymeric immunoglobulins IgM and IgA.

Joining (J) gene segment

A short gene segment that is located 3' to the V gene segments in immunoglobulin and TCR V genes and codes for part of the variable region.

K antigens

Capsular antigens of Gram-negative bacteria.

Killer cell

See Cytotoxic Cell; Natural Killer Cells.

Kinins

Vasoactive peptides produced in injured or inflamed tissue.

Kupffer cells

Macrophages lining the sinusoids of the liver.

Lactenins

Bactericidal molecules in milk.

Lag period

The interval between administration of antigen and the first detection of antibody.

Langerhans cells

Dendritic cells found in the skin. They are effective antigen-presenting cells.

Lectin

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A protein that can bind specifically to a carbohydrate. Some lectins of plant origin can induce lymphocytes to divide.

Leukemia

A cancer consisting of white cells that proliferate within the blood.

Leukocytes

White blood cells. This general term covers all the nucleated cells of blood.

Leukopenia

The absence of leukocytes.

Leukotrienes

Vasoactive metabolites of arachidonic acid produced by the actions of lipoxygenase.

Ligand

A generic term for the molecules that bind specifically to a receptor.

Linkage disequilibrium

A situation in which a pair of genes is found in a population at an unexpectedly high frequency when compared with the frequency of the individual genes. It occurs when two genes are located close to each other so that recombinations rarely occur.

Linked recognition

The necessity for lymphocytes to receive two simultaneous signals to be activated.

Locus

The location of a gene in a chromosome.

Looping out

A method of excising a segment of intervening DNA (intron) in order to join two gene segments (exons).

Lymph

The clear tissue fluid that flows through lymphatic vessels.

Lymphadenopathy

Literally, "disease of lymph nodes." In practice it is used to describe enlarged lymph nodes.

Lymphoblast

A dividing lymphocyte.

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Lymphocyte

A small mononuclear cell with a round nucleus containing densely packed chromatin found in blood and lymphoid tissues. Most have only a thin rim of cytoplasm. They recognize foreign antigens through specialized receptors.

Lymphocyte trapping

The trapping of lymphocytes within a lymph node during the node's response to antigen.

Lymphokine-activated killer cells

Lymphocytes activated by exposure to cytokines such as IL-2 in vitro.

Lymphokines

Cytokines secreted by lymphocytes.

Lymphopenia

Abnormally low numbers of lymphocytes in blood.

Lymphotoxins

Cytotoxic cytokines secreted by lymphocytes.

Lysosomal enzymes

The complex mixture of enzymes, many of which are proteases, found within lysosomes.

Lysosomes

Cytoplasmic organelles found within phagocytic cells that contain a complex mixture of potent proteases.

Lysozyme

An enzyme present in tears, saliva, and neutrophils. It attacks carbohydrates in the cell walls of Gram-positive bacteria.

Macrophages

Large phagocytic cells containing a single rounded nucleus.

Major histocompatibility complex

The gene region that contains the genes for the major histocompatibility molecules, as well as for some complement components and related proteins.

Malignant tumors

Tumors whose cells have a tendency to invade normal tissues and spread by lymphatics or blood to distant tissue sites.

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Marsupials

The order containing the pouched mammals. These include not only the Australian forms, such as kangaroos and koalas, but also the opossums.

Maternal antibodies

Antibodies that originate in the mother but enter the bloodstream of her offspring either by transport across the placenta as in primates, or by adsorption of ingested colostrum in other mammals.

Medulla

The region in the center of lymphoid organs such as the thymus or lymph nodes.

Membrane attack complex

The complement protein structure that is embedded in target cell membranes, resulting in their lysis.

Memory cells

Lymphocytes formed as a result of exposure to antigen. They have the ability to mount an enhanced response to antigen as compared with lymphocytes that have not previously encountered antigen.

Memory response

The enhanced immune response that is triggered as a result of exposing a primed animal to antigen.

Mesangial cells

Modified muscle cells found within a glomerulus.

MHC molecules

Proteins coded for by genes located in the major histocompatibility complex (see Chapter 7).

MHC restriction

The necessity for a T cell to recognize an antigen in association with an MHC molecule. It is required for helper and cytotoxic T cells to recognize antigen and for helper T cells to cooperate with B cells.

Microglia

Macrophages resident within the brain.

Mitogen

Any substance that makes cells divide.

Mixed lymphocyte reaction

Lymphocyte proliferation induced by contact with foreign lymphocytes in vitro.

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Modified live virus

A virus whose virulence has been reduced so that it can replicate in the host but cannot cause disease in normal animals.

Molecular mimicry

The development by parasites or other infectious agents of molecules whose structure closely resembles molecules found in their host. In this way the invaders may be able to evade destruction by the immune system or perhaps trigger autoimmunity.

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Monoclonal

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Originating from a single clone of cells.

Monoclonal antibody

Antibody derived from a single clone of cells and hence chemically homogeneous.

Monoclonal gammopathy

The appearance in serum of a high level of a monoclonal immunoglobulin. This is commonly, but not always, associated with the presence of a myeloma.

Monocytes

Immature macrophages found in the blood.

Monokines

Cytokines secreted by macrophages and monocytes.

Monomer

The basic unit of a molecule that can be assembled using repeating subunits.

Mononuclear cells

Those leukocytes with a single round nucleus; for example, lymphocytes and macrophages.

Mononuclear-phagocytic system

The cells that belong to the macrophage family and their precursors.

Monotremes

The order containing the least evolved egg-laying mammals. These include the platypus and the spiny anteaters (Echidna).

Myeloid system

All the granulocytes and their precursors. These precursor cells are found in the bone marrow.

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Myeloma

A tumor of plasma cells.

Myeloma protein

The immunoglobulin product secreted by a myeloma cell.

Natural antibodies

Antibodies against foreign antigens found in serum in the absence of known antigenic stimulation from immunization or an infection. Most probably arise as a result of exposure to cross-reacting bacterial antigens.

Natural killer cells

Large granular lymphocytes that are found in normal, unsensitized individuals and that can recognize and kill abnormal cells such as tumor- and virus-infected cells.

Natural suppressor cells

A population of cells found in unimmunized individuals that have the ability to suppress some immune responses.

Necrosis

Cell death due to pathological causes.

Negative feedback

A control mechanism whereby the products of a reaction act to suppress their own production.

Negative selection

The killing of T cells that have the potential to react to self-antigens. A key mechanism in the prevention of autoimmunity.

Nematode

A roundworm.

Neutralization

Blockage of the activity of an organism or a toxin by antibody.

Neutropenia

Low numbers of neutrophils in blood.

Neutrophilia

High numbers of neutrophils in blood.

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Neutrophils

Polymorphonuclear neutrophil granulocytes.

NK cells

Natural killer cells.

Noncovalent bonds

Chemical bonds, such as hydrogen or hydrophobic bonds, that can reversibly link peptide chains. They play a key role in the binding of antigen with antibodies or with T cell antigen receptors.

Normal flora

The microbial population consisting mainly of bacteria that colonize normal body surfaces. They play a key role in preventing invasion by pathogenic organisms.

Nucleocapsid

The key structural component of a virus consisting of the viral nucleic acid and its protective capsid coat.

Nude mice

A mutant strain of mice that have no thymus and are hairless.

O antigens

Somatic antigens of Gram-negative bacteria.

Obligate intracellular parasite

An organism that is absolutely required to grow inside cells. Viruses are excellent examples.

Oncofetal antigens

Antigens found on fetal and tumor cells.

Oncogene

A gene whose protein product plays a key role in cell division. As a result, its uncontrolled production leads to excessive cell growth and tumor formation. Oncogenes may be found in normal cells, as well as in cancer-causing viruses.

Oncogenic virus

A virus that causes cancer.

Ontogeny

The embryonic development of an organ or animal.

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Opportunistic pathogen

An organism that, although unable to cause disease in a healthy individual, may invade and cause disease in an individual whose immunological defenses are impaired.

Opsonin

A molecule that facilitates phagocytosis by coating foreign particles.

Optimal proportions

When antigen and antibody combine, this is the ratio of reactants that generates the largest immune complexes.

Osteichthyes

The class containing the bony fish. It includes several orders of fish, the most highly evolved of which are the teleosts. The teleosts include such typical fish as the goldfish, catfish, and trout.

Paracortex

The region located between the cortex and medulla of lymph nodes in which T cells predominate.

Paratopes

The antigen-combining sites on an immuno-globulin.

Passive agglutination

The agglutination of inert particles by antibody directed against antigen bound to their surface.

Passive immunization

Protection of one individual conferred by administration of antibody produced in another individual.

Pathogen-associated molecular pattern

Molecular structures widely distributed among pathogenic microbes.

Pathogenesis

The mechanism of a disease.

Pathogenic organism

An organism that causes disease.

Pattern recognition receptors

Cellular receptors that can selectively bind pathogen-associated molecular structures.

Perforin

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A family of proteins made by T cells and NK cells (and the complement component C9) that, when polymerized, can insert themselves into target cell membranes and provoke cell lysis.

Phagocytes

Cells whose prime function is to eat foreign particles, especially bacteria. They include macrophages and related cells, neutrophils, and eosinophils.

Phagocytosis

The ability of some cells to ingest foreign particles. Literally, “eating by cells.”

Phagolysosome

A structure produced by the fusion of a phagosome and a lysosome following phagocytosis.

Phagosome

The cytoplasmic vesicle that encloses an ingested organism.

Phenogroup

A set of blood group alleles that are consistently inherited as a group.

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Phylogeny

The evolutionary history of a plant or animal species.

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Phytohemagglutinin

A lectin derived from the red kidney bean. It acts as a T cell mitogen.

Pinocytosis

The endocytosis of small fluid droplets.

Plaque-forming cells

Antibody-secreting cells capable of forming plaques in a layer of red blood cells in the presence of complement.

Plasma

The clear fluid that forms the liquid phase of blood.

Plasma cell

A fully differentiated B cell capable of synthesizing and secreting large amounts of antibody.

Point mutation

A mutation resulting from an alteration in a single base in a gene.

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Pokeweed mitogen

A lectin derived from the pokeweed plant that stimulates T and B cells to divide.

Polyclonal gammopathies

The appearance in serum of a high level of immunoglobulins of many different specificities originating from many different clones.

Polymorphism

Inherited structural differences among proteins from allogeneic individuals as a result of multiple alternative alleles at a single locus.

Polymorphonuclear neutrophil granulocytes

Blood leukocytes possessing neutrophilic cytoplasmic granules and an irregular lobed nucleus.

Positive selection

The enhanced proliferation of cells within the thymus that can respond optimally to foreign antigen.

Precipitation

The clumping of soluble antigen molecules by antibody to reproduce a visible precipitate.

Premunition

A form of immunity seen in some parasitic conditions that depends on the continued presence of the parasite in the host.

Prevalence

The number of cases of a disease.

Primary binding tests

Serological assays that directly detect the binding of antigen and antibody.

Primary immune response

The immune response resulting from an individual's first encounter with an antigen.

Primary immunodeficiencies

Inherited immunodeficiency diseases.

Primary lymphoid organ

An organ that serves as a source of lymphocytes or in which lymphocytes mature.

Primary pathogen

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An organism that can cause disease without first suppressing an individual's immune defenses.

Primary structure

The amino acid sequence of a protein.

Privileged sites

Locations within the body where foreign grafts are not rejected. A good example is the cornea of the eye.

Programmed cell death

Physiological killing of a cell. Morphologically, these cells show apoptosis.

Prokaryotic organism

An organism composed of cells whose genetic material is free in the cytoplasm and as a result do not contain a recognizable nucleus.

Prostaglandins

Biologically active lipid metabolites of arachidonic acid produced by the actions of the enzyme cyclooxygenase.

Proteasome

A large complex enzyme structure found in the cytosol. It acts on ubiquitinated cellular proteins to cleave them into small fragments.

Protein kinase

An enzyme that phosphorylates proteins.

Protooncogene

A normal cellular gene that, when mutated, can result in a cell becoming malignant.

Prozone

The inhibition of agglutination by the presence of high concentrations of antibody.

Pseudogenes

DNA sequences that resemble functional genes but that cannot be transcribed.

Pyrogen

A fever-causing substance.

Pyroninophilic

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Stained by the dye pyronin. This stain preferentially binds to RNA. A cell whose cytoplasm stains intensely with pyronin is rich in ribosomes and is therefore probably a protein-synthesizing cell.

Radioimmunoassay

An immunological test that requires the use of an isotope-labeled reagent.

Reaginic antibody

An antibody of the IgE class that mediates type I hypersensitivity.

Recombinant vaccine

A vaccine containing antigen prepared by recombinant DNA techniques.

Respiratory burst

The rapid increase in metabolic activity that occurs in phagocytic cells while particles are being ingested. It generates potent oxidants that can kill invading microorganisms.

Reticuloendothelial system

All the cells in the body that take up circulating colloidal dyes. Many are macrophages. This term is best avoided because it is not a true body system.

Retrovirus

An RNA virus that employs the enzyme reverse transcriptase to convert its RNA into DNA.

Reverse transcriptase

An enzyme that reversely transcribes RNA to DNA. It is found in retroviruses such as FIV.

Rheumatoid factor

An autoantibody directed against epi-topes on the immunoglobulin Fc region. Classically found in the blood of patients with rheumatoid arthritis.

Rosettes

The structure formed when several red blood cells bind to the surface of another cell in suspension.

Sarcoma

A tumor arising from cells of mesodermal origin.

Secondary binding tests

Serological tests, such as agglutination and precipitation, that detect the consequences of antigen-antibody binding.

Secondary immune response

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An enhanced immune response that results from second or subsequent exposure to an antigen.

Secondary immunodeficiencies

Immunodeficiency diseases resulting from a known, nongenetic cause.

Secondary infections

Infections by organisms that can invade only a host whose defenses are first weakened or destroyed by other infectious organisms.

Secondary lymphoid organ

A lymphoid organ whose function is to trap and respond to foreign antigens.

Secondary response

The response of a sensitized animal to foreign antigen.

Secondary structure

The way in which a peptide chain is made up of structural components such as α -helices and β -pleated sheets.

Second-set reaction

The rapid rejection of an organ or tissue graft by a previously sensitized host.

Secretory component

A protein produced by mucosal epithelial cells; it functions as an IgA receptor and, on binding to IgA, protects IgA against proteases in the intestine.

Selectin

A family of cell surface adhesion proteins that bind cells to glycoproteins on vascular endothelium.

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Self-cure

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The elimination of intestinal worms by a localized type I hypersensitivity reaction in the intestinal tract.

Sensitization

The triggering of an immune response by exposure to an antigen.

Septic shock

A severe disease condition that results from the massive release of cytokines such as TNF as a result of infection with large numbers of Gram-negative bacteria.

Seroconversion

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The appearance of antibodies in blood, indicating the onset of an infection.

Serology

The science of antibody detection.

Serum

The clear, yellow fluid that is expressed when blood has clotted and the clot contracts.

Serum sickness

A type III hypersensitivity response to the administration of foreign serum as a result of the development of immune complexes in the bloodstream.

Signal transduction

The transmission of a signal through a receptor to a cell by means of a series of linked reactions.

Skin test

A diagnostic procedure that induces a local inflammatory response following intradermal inoculation of an antigen or allergen.

Somatic antigens

Antigens associated with bacterial bodies.

Somatic mutation

Mutations that occur in somatic rather than germ line cells. In immunology, this refers to the extensive mutations that occur in the V genes of B cells during the course of an immune response.

Specificity

A term that describes the ability of a test to give true positive reactions.

Splice

The joining of two DNA or RNA segments (exons) together.

Stem cell

A cell that can give rise to many different differentiated cell lines.

Stimulation index

A measure of the extent to which a cell population is stimulated to divide. It is the ratio of thymidine uptake in a stimulated cell population to the thymidine uptake in an unstimulated population.

Subclass

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Different immunoglobulin isotypes closely related within a specific class.

Subisotype

See Subclass.

Substrate modulation

A method of controlling enzyme activity seen in the complement system, by which a protein cannot be cleaved by a protease until it first binds to another protein.

Superantigen

A molecule that, as a result of its ability to bind to certain TCR variable regions, can cause certain T cells to divide.

Superfamily

A grouping of protein molecules that share common structures. For example, the members of the immunoglobulin superfamily all contain characteristic immunoglobulin domains.

Suppressor cells

Lymphocytes (usually T cells) that are claimed to suppress the response of other cells to antigen.

Syncytium

The fusion of many cells into one large cytoplasmic mass containing multiple nuclei. Usually the result of viral action.

Syndrome

A group of symptoms that together are characteristic of a specific disease.

Syngeneic (isogeneic)

Genetically identical.

T lymphocyte

A lymphocyte that has undergone a period of processing in the thymus and is responsible for mediating cell-mediated immune responses.

Tertiary binding tests

Serological tests that measure the protective ability of an antibody in living animals.

Tertiary structure

The way in which the peptide chains of a protein are folded together.

Thoracic duct

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The major lymphatic vessel that collects the lymph, draining the lower portion of the body.

Thymectomy

Surgical removal of the thymus.

Thymocytes

Developing lymphocytes in the thymus.

Thymus-dependent antigen

An antigen that requires the assistance of T helper cells to provoke an immune response.

Thymus-independent antigen

An antigen that can activate B cells and trigger an antibody response without help from T cells.

Titer

The reciprocal of the highest dilution of a serum that gives a reaction in an immunological test.

Titration

The measurement of the level of specific antibodies in a serum, achieved by testing increasing dilutions of the serum for antibody activity.

Tolerance

A state of specific unresponsiveness to an antigen induced by prior exposure to that antigen.

Tolerogen

A substance that induces tolerance.

Toxic shock

A disease resulting from exposure to large amounts of staphylococcal superantigen.

Toxoid

Nontoxic derivatives of toxins used as antigens.

Transcription

The conversion of a DNA nucleotide sequence into an RNA nucleotide sequence by complementary base pairing.

Transcription factors

Specialized proteins that regulate gene activity by binding to the promoter region of a gene. They thus turn gene transcription on or off.

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Transduction

The conversion of a signal from one form to another.

Translation

The conversion of the RNA nucleotide sequence into an amino acid sequence in a ribosome.

Transporter protein

Proteins that bind fragments of endogenous antigen and carry them to newly assembled MHC class I molecules in the endoplasmic reticulum.

Trematode

A helminth known as a fluke. Trematodes are important human and animal parasites.

Tubercle

A persistent inflammatory response to the presence of mycobacteria in the tissues.

Tuberculin

An extract of tubercle bacilli used in a diagnostic skin test for tuberculosis.

Tumor necrosis factors

Macrophage and lymphocyte-derived cytokines that can exert a direct toxic effect on neoplastic cells.

Tunicates

Complex marine invertebrates possessing characteristic outer cuticular coverings whose embryonic stages possess features that resemble those found in some vertebrates.

Tyrosine kinase

An enzyme that phosphorylates tyrosine residues in proteins. It plays a key role in signal transduction.

Urodeles

The most primitive order of amphibians; it includes the newts and salamanders.

Urticaria

Inflammatory and edematous skin reactions due to allergic mechanisms and associated with intense itching.

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Vaccination

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The administration of an antigen (vaccine) to stimulate a protective immune response against an infectious agent. The term originally referred specifically to protection against smallpox. It is synonymous with immunization.

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Vaccine

A suspension of living or inactivated organisms used as an antigen to confer immunity.

Variable region

That part of the immunoglobulin or TCR peptide chains in which the amino acid sequence shows significant variation among molecules.

Variolation

An early method of protecting an individual against smallpox by inoculation with live smallpox virus.

Vasculitis

Inflammation of blood vessel walls.

Viral interference

The inhibition of virus invasion of a cell by the presence of a competing virus or gene.

Virgin lymphocyte

A lymphocyte that has not previously encountered antigen.

Virion

A virus particle.

Virulence

The ability of an organism to cause disease.

Xenograft

A graft between two animals of different species.

Xenohybridoma

A hybridoma formed by fusing plasma cells and myeloma cells from two different species (e.g., mouse and bovine).